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(54) Title: METHOD FOR DETECTION AND TREATMENT OF BREAST CANCER

(57) Abstract

The present invention provides a method of detecting and diagnosing pre-invasive breast cancer by identifying differentially expressed genes in early, pre-invasive breast cancer tissue. Differentially expressed genes can be used as genetic markers to indicate the presence of pre-invasive cancerous tissues. Microscopically directed tissue sampling techniques combined with differential display or differential screening of cDNA libraries are used to determine differential expression of genes in the early stages of breast cancer. Differential expression of genes in pre-invasive breast cancer tissue is confirmed by RT-PCR, nuclease protection assays and in-situ hybridization of ductal carcinoma in situ tissue RNA and control tissue RNA. The present invention also provides a method of screening for compounds that induce expression of the BRCA1 gene, whose product negatively regulates cell growth in both normal and malignant mammary epithelial cells. The present invention also relates to gene therapy method using this gene.

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DESCRIPTION**"METHOD FOR DETECTION AND TREATMENT OF BREAST CANCER"**TECHNICAL FIELD

5 The present invention relates generally to methods of detection and diagnosis of breast cancer and more particularly to a diagnostic method which relies on the identification of marker genes expressed in pre-invasive cancers by microscopically-directed cloning. Furthermore, this invention concerns the prevention, detection, and diagnosis of breast cancer by addressing the molecular events which occur during the 10 earliest alterations in breast tissue.

The present invention also relates generally to methods of treatment of breast cancer, and more particularly to gene therapy methods and methods for screening compounds that induce expression of the BRCA1 gene product.

15 BACKGROUND ART

It will be appreciated by those skilled in the art that there exists a need for a more sensitive and less invasive method of early detection and diagnosis of breast cancer than those methods currently in use. Breast cancer presents inherent difficulties in regard to the ease with which it is detected and diagnosed. This is in contrast to 20 detection of some other common cancers, including skin and cervical cancers, the latter of which is based on cytomorphologic screening techniques.

There have been several attempts to develop improved methods of breast cancer detection and diagnosis. In the attempts to improve methods of detection and diagnosis of breast cancer, numerous studies have searched for oncogene mutations, gene 25 amplification, and loss of heterozygosity in invasive breast cancer (Callahan, et al., 1992; Cheickh, et al., 1992; Chen, et al., 1992; and, Lippman, et al., 1990). However, few studies of breast cancer have analyzed gene mutations and/or altered gene expression in ductal carcinoma in situ (DCIS). Investigators have demonstrated high levels of p53 protein in 13-40% of DCIS lesions employing a monoclonal antibody to 30 p53, and subsequent sequencing demonstrated mutations in several cases (Poller et al., 1992). The neu/erbB2 gene appears to be amplified in a subset of DCIS lesions (Allred et al., 1992; Maguire et al., 1992). Histologic analysis of DCIS cases suggests that mutations and altered gene expression events, as well as changes in chromatin and

DNA content, occur predominantly in comedo DCIS (Böcker et al, 1992; Killeen et al, 1991; and, Komitowski et al, 1990), which has a rapid rate of local invasion and progression to metastasis. Thus, there are presently no reliable marker genes for non-comedo DCIS (NCDCIS, hereafter).

5           Cancer in humans appears to be a multi-step process which involves progression from pre-malignant to malignant to metastatic disease which ultimately kills the patient. Epidemiologic studies in humans have established that certain pathologic conditions are "pre-malignant" because they are associated with increased risk of malignancy. There is precedent for detecting and eliminating pre-invasive lesions as a cancer prevention 10 strategy: dysplasia and carcinoma in-situ of the uterine cervix are examples of pre-malignancies which have been successfully employed in the prevention of cervical cancer by cytologic screening methods. Unfortunately, because the breast cannot be sampled as readily as cervix, the development of screening methods for breast pre-malignancy involves more complex approaches than cytomorphologic screening now 15 currently employed to detect cervical cancer.

20           Pre-malignant breast disease is also characterized by an apparent morphological progression from atypical hyperplasias, to carcinoma in-situ (pre-invasive cancer) to invasive cancer which ultimately spreads and metastasizes resulting in the death of the patient. Careful histologic examination of breast biopsies has demonstrated intermediate stages which have acquired some of these characteristics but not others. 25           Detailed epidemiological studies have established that different morphologic lesions progress at different rates, varying from atypical hyperplasia (with a low risk) to comedo ductal carcinoma-in-situ which progresses to invasive cancer in a high percentage of patients (London et al, 1991; Page et al, 1982; Page et al, 1985; Page et al, 1991; and Page et al, 1978). Family history is also an important risk factor in the development of breast cancer and increases the relative risk of these pre-malignant 30           lesions (Dupont et al, 1985; Dupont et al, 1993; and, London et al, 1991). Of particular interest is non-comedo carcinoma-in-situ which is associated with a greater than ten-fold increased relative risk of breast cancer compared to control groups (Ottesen et al, 1992; Page et al, 1982). Two other reasons besides an increased relative risk support the concept that DCIS is pre-malignant: 1) When breast cancer occurs in

these patients it regularly occurs in the same region of the same breast where the DCIS was found; and 2) DCIS is frequently present in tissue adjacent to invasive breast cancer (Ottesen et al, 1992; Schwartz et al, 1992). For these reasons DCIS very likely represents a rate-limiting step in the development of invasive breast cancer in women.

DCIS (sometimes called intraductal carcinoma) is a group of lesions in which the cells have grown to completely fill the duct with patterns similar to invasive cancer, but do not invade outside the duct or show metastases at presentation. DCIS occurs in two forms: comedo DCIS and non-comedo DCIS. Comedo DCIS is often a grossly palpable lesion which was probably considered "cancer" in the 19th and early 20th century and progresses to cancer (without definitive therapy) in at least 50% of patients within three years (Ottesen et al, 1992; Page et al, 1982). Most of the molecular alterations which have been reported in pre-malignant breast disease have been observed in cases of comedo DCIS (Poller et al, 1993; Radford et al, 1993; and, Tsuda et al, 1993). Non-comedo DCIS is detected by microscopic analysis of breast aspirates or biopsies and is associated with a 10 fold increased risk of breast cancer, which corresponds to a 25-30% absolute risk of breast cancer within 15 years (Ottesen et al, 1992; Page et al, 1982; and, Ward et al, 1992).

Widespread application of mammography has changed the relative incidence of comedo and non-comedo DCIS such that NCDCIS now represents the predominant form of DCIS diagnosed in the United States (Ottesen et al, 1992; Page et al, 1982; and Pierce et al, 1992). Both forms of DCIS generally recur as invasive cancer at the same site as the pre-malignant lesion (without definitive therapy). The precursor lesions to DCIS are probably atypical ductal hyperplasia and proliferative disease without atypia which are associated with lower rates of breast cancer development, but show further increased risk when associated with a family history of breast cancer (Dupont et al, 1985; Dupont et al, 1989; Dupont et al, 1993; Lawrence, 1990; London et al, 1991; Page et al, 1982; Page et al, 1985; Page et al, 1991; Page et al, 1978; Simpson et al, 1992; Solin et al, 1991; Swain, 1992; Weed et al, 1990).

What is needed, then, is a sensitive method of detection and diagnosis of breast cancer when the cancerous cells are still in the pre-invasive stage. To illustrate the usefulness in early breast cancer detection of a marker gene and its encoded protein,

consider the dramatic impact that prostate specific antigen has had on early stage prostate cancer. This method of early detection and diagnosis of breast cancer is presently lacking in the prior art.

Breast cancer occurs in hereditary and sporadic forms. Recently the BRCA 1 gene has been cloned and shown to be mutated in kindreds with hereditary breast and ovarian cancer (Hall et al. 1990, Miki, Y. et al. 1994, Friedman et al. 1994, Castilla et al. 1994, Simard et al. 1994). Although 92% of families with two or more cases of early-onset breast cancer and two cases of ovarian cancer have germ-line mutations in BRCA 1 (Narod et al. in press), the gene has not been shown to be mutated in any truly sporadic case to date (Futreal et al. 1994). Despite the surprising paucity of somatically acquired mutations in sporadic breast cancer, it is still a likely tumor suppressor gene with a key role in breast epithelial cell biology. The BRCA 1 gene encodes a protein of 1863 amino acids with a predicted zinc finger domain observed in proteins which regulate gene transcription. Until the discovery of the function of the BRCA1 gene in conjunction with the development of the present invention, the function was unknown.

#### DISCLOSURE OF THE INVENTION

Epidemiologic studies have established that NCDCIS of the breast is associated with a ten-fold increased risk of breast cancer (absolute risk of 25-30%). It seems likely that this pre-invasive lesion is a determinate precursor of breast cancer because the subsequent development of breast cancer is regularly in the same region of the same breast in which the NCDCIS lesion was found. Important aspects of the present invention concern isolated DNA segments and those isolated DNA segments inserted into recombinant vectors encoding differentially expressed marker genes in abnormal tissue, specifically in NCDCIS, as compared with those expressed in normal tissue, and the creation and use of recombinant host cells through the application of DNA technology, which express these differentially expressed marker genes (Sambrook et al, 1989).

Because there are no cell lines or animal models which clearly display known characteristics of pre-invasive breast disease, human breast tissue samples are essential

for studying pre-invasive breast disease. Using human tissue samples, we subsequently have developed a method for cDNA cloning from histologically identified lesions in human breast biopsies. We have used this method to clone genes which are differentially expressed in pre-invasive breast lesions such as NCDCIS lesions as compared to genes expressed in normal tissue. The differentially expressed genes detected in pre-invasive breast cancer are called marker genes. Identification of marker genes for pre-invasive breast disease provides improved methods for detection and diagnosis of pre-invasive breast cancer tissue, and further provides marker genes for studies of the molecular events involved in progression from pre-invasive to malignant breast disease.

Analysis of marker gene expression in NCDCIS presents the advantage that cancerous breast tissue at that stage is non-invasive. Detection and diagnosis of NCDCIS by means of differentially expressed marker genes compared to the same marker genes in normal breast tissue, would allow a greater ability to detect, prevent and treat the disease before it becomes invasive and metastasizes. The stage or intermediate condition of NCDCIS is a particularly good candidate for early intervention because it is 1) prior to any invasion and thus prior to any threat to life; 2) it is followed by invasive carcinoma in over 30% of cases if only treated by biopsy; and, 3) there is a long "window" of opportunity (4-8 years) approximately before invasive neoplasia occurs. Thus, NCDCIS is an ideal target for early diagnosis. While these morphologically defined intermediate endpoints have been widely accepted, progress in defining the molecular correlates of these lesions has been hampered by an inability to identify and sample them in a manner which would allow the application of molecular techniques.

Frozen tissue blocks from breast biopsies were used to construct and screen cDNA libraries prepared from NCDCIS tissue, normal breast tissue, breast cancer tissue, and normal human breast epithelial cells. Several cDNAs which were differentially expressed in human DCIS epithelial cells compared to normal breast epithelial cells were cloned and sequenced. One gene which is differentially expressed is the M2 subunit of RibRed which is expressed at low levels in human breast epithelial cells but at higher levels in 4 out of 5 DCIS tissue samples. It is presumed that the

altered morphologic appearance and determinant biologic behavior of DCIS results from altered expression of genes (such as RibRed) which is important in the induction of breast cancer in humans.

This invention, therefore, provides a method of detecting and diagnosing pre-invasive breast cancer by analyzing marker genes which are differentially expressed in non-comedo DCIS cells. Histopathologic studies have demonstrated that these morphologic patterns in breast tissue lead to invasive breast cancer in at least 20-30% of patients. The present method analyzes gene expression in normal, pre-malignant and malignant breast biopsies; and, it allows simultaneous comparison and cloning of marker genes which are differentially expressed in pre-invasive breast cancer. These marker genes can then be used as probes to develop other diagnostic tests for the early detection of pre-invasive breast cancer.

The present invention concerns DNA segments, isolatable from both normal and abnormal human breast tissue, which are free from total genomic DNA. The isolated DCIS-1 protein product is the regulatory element of the RibRed enzyme. This and all other isolatable DNA segments which are differentially expressed in preinvasive breast cancer can be used in the detection, diagnosis and treatment of breast cancer in its earliest and most easily treatable stages. As used herein, the term "abnormal tissue" refers to pre-invasive and invasive breast cancer tissue, as exemplified by collected samples of non-comedo or comedo DCIS tissues.

As used herein, the term "DNA segment" refers to a DNA molecule which has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a differentially expressed protein (as measured by the expression of mRNA) in abnormal tissue refers to a DNA segment which contains differentially expressed-coding sequences in abnormal tissue as compared to those expressed in normal tissue, yet is isolated away from, or purified free from, total genomic DNA of *Homo sapiens sapiens*. Furthermore, a DNA segment encoding a BRCA1 protein refers to a DNA segment which contains BRCA1 coding sequences, yet is isolated away from, or purified free from, total genomic DNA of *Homo sapiens sapiens*. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids,

phage, viruses, and the like.

Similarly, a DNA segment comprising an isolated or purified differentially expressed gene or comprising an isolated or purified BRCA1 gene refers to a DNA segment including differentially expressed coding sequences or BRCA1 coding sequences isolated substantially away from other naturally occurring genes or protein encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein, polypeptide or peptide encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences and cDNA sequences. "Isolated substantially away from other coding sequences" means that the gene of interest, in this case, any differentially expressed marker gene or the BRCA1 gene, forms the significant part of the coding region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode differentially expressed genes in pre-invasive breast cancer, each which includes within its amino acid sequence an amino acid sequence in accordance with SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7, all seq id no:s 1-7 are derived from non-comedo DCIS samples from *Homo sapiens sapiens*. In other particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences which encode the M2 subunit of human RibRed that includes within its amino acid sequence the similar amino acid sequence of hamster RibRed corresponding to the M2 subunit of hamster RibRed.

In certain embodiments, the invention concerns isolated DNA segments and recombinant vectors which partially or wholly encode a protein or peptide that includes within its amino acid sequence an amino acid sequence essentially as partially or wholly encoded, respectively, by SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7. Naturally, where the DNA segment or vector encodes a full length differentially expressed protein, or is intended

for use in expressing the differentially expressed protein, the most preferred sequences are those which are essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7 and which encode a protein that exhibits differential expression, e.g., as may be determined by the differential display or differential sequencing assay, as disclosed herein.

The term "a sequence essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7" means that the sequence substantially corresponds to a portion of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7, respectively, and has relatively few nucleotides which are not identical to, or a biologically functional equivalent of, the nucleotides of the respective SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein, for example see pages 24 through 25. Accordingly, sequences which have between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids which are identical or functionally equivalent to the amino acids of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7 will be sequences which are "essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7", respectively..

In particular embodiments, the invention concerns a drug screening method and a gene therapy method that use isolated DNA segments and recombinant vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence an amino acid sequence in accordance with SEQ ID NO:49, SEQ ID NO:49 derived from breast tissue from *Homo sapiens*. In other particular embodiments, the invention concerns isolated DNA sequences and recombinant DNA vectors incorporating DNA sequences which encode a protein that includes within its amino acid sequence the amino acid sequence of the BRCA1 gene product from human breast tissue.

In certain embodiments, the invention concerns methods using isolated DNA segments and recombinant vectors which partially or wholly encode a protein or peptide that includes within its amino acid sequence an amino acid sequence essentially as set forth in SEQ ID NO:49. Naturally, where the DNA segment or vector encodes a full length BRCA1 protein, or is intended for use in expressing the BRCA1 protein, the most preferred sequences are those which are essentially as set forth in SEQ ID NO:47 and which encode a protein that retains activity as a negative growth regulator in human breast cells, as may be determined by antisense assay, as disclosed herein.

The term "a sequence essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7" means that the sequence substantially corresponds to a portion of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7, respectively, and has relatively few nucleotides which are not identical to, or a biologically functional equivalent of, the nucleotides of the respective SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein, for example see pages 24 through 25. Accordingly, sequences which have between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99%; of amino acids which are identical or functionally equivalent to the amino acids of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7 will be sequences which are "essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7", respectively.

The term "a sequence essentially as set forth in SEQ ID NO:49" means that the sequence substantially corresponds to a portion of SEQ ID NO:49 and has relatively few amino acids which are not identical to, or a biologically functional equivalent of, the nucleotides of SEQ ID NO:49. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein, for example see pages 24 through 25. Accordingly, sequences which have between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more

preferably, between about 91% and about 99%; of amino acids which are identical or functionally equivalent to the amino acids of SEQ ID NO:49 will be sequences which are "essentially as set forth in SEQ ID NO:49".

5 In certain other embodiments, the invention concerns isolated DNA segments and recombinant vectors that include within their sequence a nucleic acid sequence essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7. The term "essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7" is used in the same sense as described above and means that the nucleic acid sequence substantially corresponds to a portion of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, respectively, and has relatively few codons which are not identical, or functionally equivalent, to the codons of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, respectively. Again, DNA segments which encode proteins exhibiting differential expression will be most preferred. The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Figure 8).

20 In certain other embodiments, the invention concerns a method for screening drugs and a gene therapy method which involve the use of isolated DNA segments and recombinant vectors that include within their sequence a nucleic acid sequence essentially as set forth in SEQ ID NO:47 and SEQ ID NO:48. The term "essentially as set forth in SEQ ID NO:47 and SEQ ID NO:48" is used in the same sense as described above and means that the nucleic acid sequence substantially corresponds to a portion of SEQ ID NO:47 and SEQ ID NO:48 respectively, and has relatively few codons which are not identical, or functionally equivalent, to the codons of SEQ ID NO:47 and SEQ ID NO:48, respectively. Again, DNA segments which encode proteins exhibiting the negative regulatory activity of the BRCA1 will be most preferred. The term "functionally equivalent codon" is used herein to refer to codons

that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Figure 8).

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences which may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, i.e., introns, which are known to occur within genes.

Excepting intronic or flanking regions, and allowing for the degeneracy of the genetic code, sequences which have between about 20% and about 50%; or more preferably, between about 50% and about 70%; or even more preferably, between about 70% and about 99%; of nucleotides which are identical to the nucleotides of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 will be sequences which are "essentially as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7", respectively. Sequences which are essentially the same as those set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 may also be functionally defined as sequences which are capable of hybridizing to a nucleic acid segment containing the complement of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, respectively, under relatively stringent conditions. Suitable relatively stringent hybridization conditions will be well known to those of skill in the art (Sambrook et al, 1989).

Excepting intronic or flanking regions, and allowing for the degeneracy of the genetic code, sequences which have between about 20% and about 50%; or more preferably, between about 50% and about 70%; or even more preferably, between about 70% and about 99%; of nucleotides which are identical to the nucleotides of SEQ ID NO:47 and SEQ ID NO:48 will be sequences which are "essentially as set forth in

SEQ ID NO:47 and SEQ ID NO:48", respectively. Sequences which are essentially the same as those set forth in SEQ ID NO:47 and SEQ ID NO:48 may also be functionally defined as sequences which are capable of hybridizing to a nucleic acid segment containing the complement of SEQ ID NO:47 and SEQ ID NO:48, respectively, under relatively stringent conditions. Suitable relatively stringent hybridization conditions will be well known to those of skill in the art (Sambrook et al, 5 1989).

It is also important to understand the molecular events which lead to progression from pre-invasive to invasive breast cancer. Breast cancer is a disease that is presumed 10 to involve a series of genetic alterations that confer increasing growth independence and metastatic capability on somatic cells. Identifying the molecular events that lead to the initial development of a neoplasm is therefore critical to understanding the fundamental mechanisms by which tumors arise and to the selection of optimal targets for gene 15 therapy and chemopreventive agents. As intermediate endpoints in neoplastic development, some pre-malignant breast lesions represent important, and possibly rate-limiting steps in the progression of human breast cancer, and careful epidemiological studies have established the relative risk for breast cancer development 20 for specific histologic lesions. In particular, invasive breast cancer develops in the region of the previous biopsy site in at least 25-30% of patients following diagnosis of non-comedo DCIS providing strong evidence that this pre-malignant lesion is a determinant event in breast cancer progression. While these morphologically defined 25 intermediate endpoints have been widely accepted, progress in defining the molecular correlates of these lesions has been hampered by an inability to identify and sample them in a manner which would allow the application of molecular techniques.

The present invention includes a comparison of gene expression between multiple breast tissue biopsy samples as a means to identify differentially expressed genes in pre-malignant breast disease compared with normal breast tissue. These 30 genetic markers should be extremely useful reagents for early diagnosis of breast cancer, and for the delineation of molecular events in progression of breast cancer.

Identification of gene markers which are expressed in the majority of pre-invasive breast cancer tissue samples involves cDNA library preparation from both

normal and abnormal tissue. This is followed by either a modified differential display method or a differential screening method to identify differential expression of genes which is subsequently confirmed by RT-PCR, nuclease protection assays and in situ hybridization of DCIS tissue RNA and control tissue RNAs (Sambrook et al, 1989).  
5 Use of genetic engineering methods can bias the screening to specifically identify genes whose encoded proteins are secreted or are present at the cell surface, in order to find proteins which will be useful markers for diagnostic blood tests (secreted proteins) or for diagnostic imaging studies (cell surface proteins).

Thus, the method of the present invention begins with the collection of at least  
10 one tissue sample by a microscopically-directed collection step in which a punch biopsy is obtained exclusively from abnormal tissue which exhibits histological or cytological characteristics of pre-invasive breast cancer. Preferably, the sample site will be an isolatable tissue structure, such as ductal epithelial cells from pre-invasive breast cancer tissue. The mRNA is purified from the sample. Then, a cDNA library is  
15 prepared from the mRNA purified from the abnormal tissue sample (Sambrook et al, 1989).

A normal tissue sample is then obtained from the patient, using a sample site from an area of tissue which does not exhibit histological or cytological characteristics of pre-invasive cancer. A cDNA library is also prepared from this normal tissue  
20 sample.

The abnormal tissue cDNA library can then be compared with the normal tissue cDNA library by differential display or differential screening to determine whether the expression of at least one marker gene in the abnormal tissue sample is different from the expression of the same marker gene in the normal tissue sample.

25 Further diagnostic steps can be added to the method by cloning the marker gene using sequence-based amplification to create a cloned marker gene which can then be DNA-sequenced in order to derive the protein sequence. The protein sequence is then used to generate antibodies which will recognize these proteins by antibody recognition of the antigen. The presence of the antibody-recognized antigen can then be detected  
30 by means of conventional medical diagnostic tests.

This invention also includes methods of screening for compounds and gene therapy methods using the BRCA1 gene. BRCA1 mRNA is expressed at 5-10 fold higher levels in normal mammary tissue than in invasive breast cancer samples. Having demonstrated that mRNA expression levels of BRCA1 are higher in normal mammary cells than in cancer cells, antisense methods were used to test the hypothesis that BRCA1 expression inhibits cell growth. These tests showed that diminished expression of BRCA1 increased the proliferative rate of breast cells.

An object of the present invention, then, is to provide a method of early detection of pre-invasive breast cancer in human tissue.

It is a further object of this invention to identify early marker genes for pre-invasive breast disease which can be used in screening methods for early pre-invasive breast cancer.

It is also an object of this invention to produce a cDNA library from pre-invasive breast cancer tissue resulting in a permanent genetic sample of that pre-invasive breast cancer tissue.

It is also an object of this invention to provide a drug or biological screening method using the BRCA 1 promoter region and gene therapy method using the BRCA 1 gene.

#### List of Abbreviations

20	TPA	Phorbol 12-myristate 13-acetate
	MCF-7	An immortalized cell line derived from a metastasis of human breast cancer
	HMEC	A primary (non-immortalized) cell line derived from breast epithelial cells obtained during reduction mammoplasty
25	DCIS	Ductal Carcinoma-in-situ
	NCDC	Non-Comedo Ductal Carcinoma in situ
	cDNA	Complementary DNA obtained from an RNA template
	DNA	Deoxyribonucleic Acid
30	RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
	RibRed	Ribonucleotide Reductase

Fig. 1 shows Table I which describes anatomic lesion types in the human breast with pre-malignant implication.

Fig. 2 shows a model for pre-malignant conditions, highlighting magnitude of risk for progression to clinical malignancy.

Fig. 3 contains color photos of DCIS tissue, before (upper left panel) and after microscopically-directed excisional punch biopsy (upper right panel). The lower panels show tissue samples of normal breast tissue (lower left panel), and invasive breast cancer (lower right panel).

Fig. 4 shows expression of collagen III mRNA in tissue mRNA samples, analyzed by RNase protection assay methods.

Fig. 5 shows differential display of cDNAs obtained from patient tissue samples and controls.

Fig. 6 shows a comparison of the sequence between DCIS-1 and the human and hamster genes.

Fig. 7 shows expression of DCIS-1 mRNA in tissue mRNA samples analyzed by RNase protection assay as described in the legend to Figure 4.

Fig. 8 is Table II which displays the genetic code.

Fig. 9 is a Table which lists differentially expressed marker genes.

Figs. 10A and 10B shows expression of BRCA1 mRNA during breast cancer progression by PCR detection and nuclease protection assay, respectively.

Figs. 11A and 11B is a comparison of BRCA1 expression in normal breast and invasive breast cancer using nuclease protection assay of RNA, respectively.

Figs. 12A, 12B, and 12C show that antisense inhibition of BRCA1 accelerates mammary cell proliferation.

Figs. 13A and 13B includes a Northern blot of mRNA and nuclear runon studies that show that ribonucleotide reductase M2 mRNA is cell cycle regulated in MCF-7 cells.

Fig. 14 includes a nuclease protection assay that shows that antisense inhibition of BRCA1 in human mammary cells decreases BRCA1 mRNA and increases ribonucleotide reductase mRNA.

**UTILITY STATEMENT**

The detection of differentially expressed genes in pre-invasive breast tissue, specifically in non-comedo ductal carcinoma in situ as compared to genes expressed in normal tissue, is useful in the diagnosis, prognosis and treatment of human breast cancer. Such differentially expressed genes are effective marker genes indicating the significantly increased risk of breast cancer in a patient expressing these differentially expressed marker genes. These marker genes are useful in the detection, early diagnosis, and treatment of breast cancer in humans.

The discovery of the function of the BRCA 1 gene has broad utility including, in the present invention, development of methods to treat familial and sporadic breast cancers as well as screen for therapeutic drugs through production of important indicator compounds.

**ACTIVITY STATEMENT**

Of the differentially expressed genes described in this invention, DCIS-1 encodes a gene similar to the M2 subunit of hamster ribonucleotide reductase. The M2 subunit of ribonucleotide reductase (RibRed, hereafter) is responsible for regulation of RibRed. The differential levels of expression of the marker genes described in this invention (Seq ID No.s 1-7), indicate genetic changes which have been linked to the presence of pre-invasive breast cancer.

The BRCA1 gene (Seq. ID No. 47) is differentially expressed in invasive breast cancer cells. The BRCA1 gene product is a negative regulator of mammary cell proliferation which is expressed at diminished levels in sporadic breast cancer.

**BEST MODE FOR CARRYING OUT THE INVENTION**

For the purposes of the subsequent description, the following definitions will be used:

Nucleic acid sequences which are "complementary" are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. That is, that the larger purines will always base pair with the smaller pyrimidines to form only combinations of Guanine paired with Cytosine (G:C) and Adenine paired with

either Thymine (A:T) in the case of DNA or Adenine paired with Uracil (A:U) in the case of RNA.

5 "Hybridization techniques" refer to molecular biological techniques which involve the binding or hybridization of a probe to complementary sequences in a polynucleotide. Included among these techniques are northern blot analysis, southern blot analysis, nuclease protection assay, etc.

10 "Hybridization" and "binding" in the context of probes and denatured DNA are used interchangeably. Probes which are hybridized or bound to denatured DNA are aggregated to complementary sequences in the polynucleotide. Whether or not a particular probe remains aggregated with the polynucleotide depends on the degree of complementarity, the length of the probe, and the stringency of the binding conditions. The higher the stringency, the higher must be the degree of complementarity and/or the longer the probe.

15 "Probe" refers to an oligonucleotide or short fragment of DNA designed to be sufficiently complementary to a sequence in a denatured nucleic acid to be probed and to be bound under selected stringency conditions.

20 "Label" refers to a modification to the probe nucleic acid that enables the experimenter to identify the labeled nucleic acid in the presence of unlabeled nucleic acid. Most commonly, this is the replacement of one or more atoms with radioactive isotopes. However, other labels include covalently attached chromophores, fluorescent moieties, enzymes, antigens, groups with specific reactivity, chemiluminescent moieties, and electrochemically detectable moieties, etc.

25 "Marker gene" refers to any gene selected for detection which displays differential expression in abnormal tissue as opposed to normal tissue. It is also referred to as a differentially expressed gene.

"Marker protein" refers to any protein encoded by a "marker gene" which protein displays differential expression in abnormal tissue as opposed to normal tissue.

"Tissuemizer" describes a tissue homogenization probe.

30 "Abnormal tissue" refers to pathologic tissue which displays cytologic, histologic and other defining and derivative features which differ from that of normal

tissue. This includes in the case of abnormal breast tissue, among others, pre-invasive and invasive neoplasms.

"Normal tissue" refers to tissue which does not display any pathologic traits.

5 "PCR technique" describes a method of gene amplification which involves sequenced-based hybridization of primers to specific genes within a DNA sample (or library) and subsequent amplification involving multiple rounds of annealing, elongation and denaturation using a heat-stable DNA polymerase.

10 "RT-PCR" is an abbreviation for reverse transcriptase-polymerase chain reaction. Subjecting mRNA to the reverse transcriptase enzyme results in the production of cDNA which is complementary to the base sequences of the mRNA. Large amounts of selected cDNA can then be produced by means of the polymerase chain reaction which relies on the action of heat-stable DNA polymerase produced by Thermus aquaticus for its amplification action.

15 "Microscopically-directed" refers to the method of tissue sampling by which the tissue sampled is viewed under a microscope during the sampling of that tissue such that the sampling is precisely limited to a given tissue type, as the investigator requires. Specifically, it is a collection step which involves the use of a punch biopsy instrument. This surgical instrument is stereotactically manually-directed to harvest exclusively from abnormal tissue which exhibits histologic or cytologic characteristics of pre-invasive cancer. The harvest is correlated with a companion slide, stained to recognize the target tissue.

20 "Differential display" describes a method in which expressed genes are compared between samples using low stringency PCR with random oligonucleotide primers.

25 "Differential screening" describes a method in which genes within cDNA libraries are compared between two samples by differential hybridization of cDNAs to probes prepared from each library.

30 "Nuclease protection assay" refers to a method of RNA quantitation which employs strand specific nucleases to identify specific RNAs by detection of duplexes.

"Differential expression" describes the phenomenon of differential genetic expression seen in abnormal tissue in comparison to that seen in normal tissue.

"Isolatable tissue structure" refers to a tissue structure which when visualized microscopically or otherwise is able to be isolated from other different surrounding tissue types.

5 "In situ hybridization of RNA" refers to the use of labeled DNA probes employed in conjunction with histological sections on which RNA is present and with which the labeled probe can hybridize allowing an investigator to visualize the location of the specific RNA within the cell.

"Comedo DCIS cells" refers to cells comprising an in situ lesion with the combined features of highest grade DCIS.

10 "Non-comedo DCIS cells" refers to cells of DCIS lesions without comedo features.

"Cloning" describes separation and isolation of single genes.

"Sequencing" describes the determination of the specific order of nucleic acids in a gene or polynucleotide.

15 The present invention provides a method for detecting and diagnosing cancer by analyzing marker genes which are differentially expressed in early, pre-invasive breast cancer, specifically in non-comedo DCIS cells. Our histopathologic studies have demonstrated that certain morphologic patterns in breast tissue are pre-malignant, leading to invasive breast cancer in at least 20-30% of patients. We have developed a new method for analyzing gene expression in normal, pre-malignant and malignant breast biopsies which allows simultaneous comparison and cloning of marker genes which are differentially expressed in pre-invasive breast cancer. These marker genes (which appear as differentially expressed genes in pre-invasive breast cancer) can be used as probes to develop diagnostic tests for the early detection of pre-invasive breast cancer (Sambrook, 1989).

20 The present invention thus comprises a method of identification of marker genes which are expressed in the majority of pre-invasive breast cancer tissue samples. It involves cDNA library preparation followed by a modified differential display method. Use of genetic engineering methods (Sambrook, 1989) can bias the screening to 30 specifically identify genes whose encoded proteins are secreted or are present at the cell

surface, in order to find proteins which will be useful markers for diagnostic blood tests (secreted proteins) or for diagnostic imaging studies (cell surface proteins).

Naturally, the present invention also encompasses DNA segments which are complementary, or essentially complementary, to the sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:47 and SEQ ID NO:48. Nucleic acid sequences which are "complementary" are those which are capable of base-pairing according to the standard Watson-Crick complementarity rules. As used herein, the term "complementary sequences" means nucleic acid sequences which are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of hybridizing to the nucleic acid segment of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:47 and SEQ ID NO:48 under relatively stringent conditions such as those described herein.

The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments may be prepared which include a short stretch complementary to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:47 and SEQ ID NO:48, such as about 10 nucleotides, and which are up to 10,000 or 5,000 base pairs in length, with segments of 500 being preferred in most cases. DNA segments with total lengths of about 1,000, 500, 200, 100 and about 50 base pairs in length are also contemplated to be useful.

It will also be understood that this invention is not limited to the particular nucleic acid and amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:47, SEQ ID NO:48, and SEQ ID NO:49. Recombinant vectors and isolated DNA

segments may therefore variously include the differentially expressed coding regions or the BRCA1 coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides which nevertheless include differentially expressed-coding regions and the BRCA1 coding regions or may encode biologically functional equivalent proteins or peptides which have variant amino acids sequences.

The DNA segments of the present invention encompass biologically functional equivalent differentially expressed proteins and peptides biologically functional equivalent proteins of BRCA1. Such sequences may arise as a consequence of codon redundancy and functional equivalency which are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, e.g., to introduce improvements to the antigenicity of the protein or to test site-directed mutants or others in order to examine carcinogenic activity of the differentially expressed marker genes at the molecular level.

If desired, one may also prepare fusion proteins and peptides, e.g., where the differentially expressed marker gene coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (e.g., proteins which may be purified by affinity chromatography and enzyme label coding regions, respectively).

Recombinant vectors form important further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment is positioned under the control of a promoter. The promoter may be in the form of the promoter which is naturally associated with a RIBRED gene, e.g., in human cells, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR technology, in connection with the compositions disclosed herein.

In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a differentially expressed marker gene or the BRCA1 gene in its natural environment. Such promoters may include MMTV promoters normally associated with other genes, and/or promoters isolated from any other bacterial, viral, eukaryotic, or mammalian cell. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of molecular biology, for example, see Sambrook *et al.* (1989). The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides.

Appropriate promoter systems contemplated for use in high-level expression include, but are not limited to appropriate bacterial promoters.

As mentioned above, in connection with expression embodiments to prepare recombinant differentially expressed marker gene encoded proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire differentially expressed protein or subunit being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the expression of differentially expressed peptides or epitopic core regions, such as may be used to generate anti-marker protein antibodies, also falls within the scope of the invention (Harlow *et al.*, 1988).

DNA segments which encode peptide antigens from about 15 to about 50 amino acids in length, or more preferably, from about 15 to about 30 amino acids in length are contemplated to be particularly useful. The C terminus of proteins provide an excellent region for peptide antigen recognition (Harlow *et al.*, 1988). DNA segments encoding peptides will generally have a minimum coding length in the order of about 45 to about 147, or to about 90 nucleotides. DNA segments encoding partial length peptides may have a minimum coding length in the order of about 50 nucleotides for

a polypeptide in accordance with seq id no:3, or about 264 nucleotides for a polypeptide in accordance with SEQ ID NO: 1.

In addition to their use in directing the expression of the differentially expressed marker proteins, the nucleic acid sequences disclosed herein also have a variety of other uses. For example, they also have utility as probes or primers in nucleic acid hybridization embodiments. As such, it is contemplated that oligonucleotide fragments corresponding to the sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 for stretches of between about 10 to 15 nucleotides and about 20 to 30 nucleotides will find particular utility. Longer complementary sequences, e.g., those of about 40, 50, 100, 200, 500, 1000, and even up to full length sequences of about 2,000 nucleotides in length, will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to differentially expressed marker gene sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having stretches of 20, 30, 50, or even of 500 nucleotides or so, complementary to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 are particularly contemplated as hybridization probes for use in, e.g., Southern and Northern blotting. This would allow differentially expressed structural or regulatory genes to be analyzed, both in patients and sample tissue from pre-invasive and invasive breast tissue. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the complementary region may be varied, such as between about 10 and about 100 nucleotides, but larger complementary stretches of up to about 300 nucleotides may be used, according to the length complementary sequences one wishes to detect.

### Nucleic Acid Hybridization

The use of a hybridization probe of about 10 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 nucleotides, or even longer where desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequences set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 and to select any continuous portion of one of the sequences, from about 10 nucleotides in length up to and including the full length sequence, that one wishes to utilise as a probe or primer. The choice of probe and primer sequences may be governed by various factors, such as, by way of example only, one may wish to employ primers from towards the termini of the total sequence, or from the ends of the functional domain-encoding sequences, in order to amplify further DNA; one may employ probes corresponding to the entire DNA, or to the 5' region, to clone marker-type genes from other species or to clone further marker-like or homologous genes from any species including human; and one may employ randomly selected, wild-type and mutant probes or primers with sequences centered around the RibRed M2 subunit encoding sequence to screen DNA samples for differentially expressed levels of RibRed, such as to identify human subjects which may be expressing differential levels of RibRed and thus may be susceptible to breast cancer.

The process of selecting and preparing a nucleic acid segment which includes a sequence from within SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7 may alternatively be described as "preparing a nucleic acid fragment". Of course, fragments may also be obtained by other techniques such as, e.g., by mechanical shearing or by restriction enzyme digestion. Small nucleic acid segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly

practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR technology of U.S. Patent 4,603,102 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

Accordingly, the nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of differentially expressed marker genes or cDNAs. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, e.g., one will select relatively low salt and/or high temperature conditions, such as provided by 0.02M-0.15M NaCl at temperatures of 50°C to 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating specific differentially expressed marker genes.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate marker gene sequences from related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as 0.15M-0.9M salt, at temperatures ranging from 20°C to 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe, etc.). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. (Sambrook et al, 1989).

In a preferred embodiment of the method, certain preliminary procedures are necessary to prepare the sample tissue and the probes before the detection of differential expression of marker genes in abnormal tissue as compared to that in normal tissue can be accomplished.

## SAMPLE PREPARATION

### RNA purification

RNA was isolated from frozen tissue samples by mincing of microdissected frozen tissue fragments with a razor blade and then adding 800 microliter of 5.6M

guanidinium to increase mixing, followed by a 30 second microcentrifuge centrifugation at 14,000 rpm to remove particulate matter. The supernatant was then removed and the viscosity was reduced by multiple aspirations through a 22 gauge needle and then 200  $\mu$ l of chloroform was added and the sample was incubated on ice for 15 minutes (during this time the sample was vortexed multiple times). Following incubation with chloroform, the sample was centrifuged for 15 minutes at 14,000 rpm and the aqueous layer was removed and ethanol precipitated. This extraction method produces RNA which is primarily derived from cells of epithelial origin. In order to obtain RNA samples which presumably includes RNA derived from these stromal cells; the particulate material (remaining in the pellet from the 30 second centrifugation) was homogenized with a tissuemizer, washed with PBS, treated with collagenase at 37°C for 30 minutes, sonicated, extracted with phenol/chloroform and ethanol precipitated.

cDNA libraries were constructed in lambda phage using polyA-selected mRNA from the following samples; cultured human breast epithelial cells, tissue from three reduction mammoplasty patients, tissue from three DCIS patients, and tissue from one DCIS patient (patient #10) that showed a focus of microinvasion adjacent to an area of DCIS. Multiple punches were needed to obtain sufficient RNA for polyA selection and library construction. 200 ug of total RNA was obtained by pooling 20 punches from normal breast tissue (reduction mammoplasty samples) and 5-8 punches from DCIS lesions, presumably reflecting the greater cellularity of the DCIS samples. cDNA libraries were constructed by first and second strand cDNA synthesis followed by the addition of directional synthetic linkers (ZAP-cDNA Synthesis Kit, Stratagene, La Jolla, California). The Xho I-Eco RI linker cDNA was then ligated into lambda arms, packaged with packaging extracts, and then used to infect XL1-blue bacteria resulting in cDNA libraries.

#### PROBE PREPARATION

The collagen III probe employed for nuclease protection assays was constructed by subcloning the 208 bp Hinc II-Pst I fragment from the 3' untranslated region of the human type III procollagen gene into pGem4Z. This region of the human procollagen III gene was obtained by PCR amplification of published sequence (Ala-Kokko et al., 1989) followed by restriction with Hinc II and Pst I. For a control probe to assure

equal loading and recovery of RNA, we used a T7 polymerase-generated probe for human glyceraldehyde phosphate dehydrogenase (GADP) which protects a 140 bp Sac I-Xba I fragment; (a generous gift from Janice Nigro, Vanderbilt University). Probe DCIS-1 was generated by linearizing the rescued plasmid with Pvu II, which should generate a 200 bp protected fragment. RNase protection assays were performed with 1 ug of unselected RNA and the above-cited probes using the methods we have reported previously (Holt, 1993).

5 Differential Display-based cloning of cDNAs:

10 Rescued cDNA library samples were used as templates for low stringency PCR with the either a pair of 25 bp primers or an anchored 14 bp primer paired with a random 25 bp primer. Random 25 bp primers were generated by a computer-based algorithm (Jotte and Holt, unpublished). Samples were denatured for two minutes at 95°C followed by 40 cycles, each cycle consisting of denaturation for 1 minute at 94°C., annealing for 2 minutes at 25°C., and extension for 1 minute at 72°C. The 15 samples were then run on an 6% non-denaturing polyacrylamide gel, which was dried and autoradiographed. Specific bands were excised then reamplified with the same primers used for their generation. Specificity was confirmed on 6% polyacrylamide gel, and samples were purified by ethanol precipitation of the remainder of the PCR reaction. Fragments were then individually cloned into SrfI cut vectors by standard 20 methods using PCR-Script<sup>TM</sup>SK(+) Cloning Kit (Stratagene, LaJolla, California) and then sequenced.

### EXAMPLE 1

#### Studies showing Increased Risk of Breast Cancer 25 in Patients with DCIS

Since the 1970's, studies of pre-invasive lesions associated with the development of breast cancer have been undertaken in an attempt to refine histologic and cytologic criteria for the hyperplastic lesions analogous to those of the uterine cervix and colon. Because of the availability of tissue from breast biopsies done many years previously, cohorts of women who underwent breast biopsies 15 to 20 years ago, can be studied to determine the risk for development of breast cancer attributable to specific lesions.

Many concurrent studies evaluating lesions associated with cancer at time of cancer diagnosis led the way in pointing out lesions of potential interest (Wellings et al, 1975). Hopefully, these intermediate stages in cancer development will serve to provide indicators of breast cancer development sufficiently precise to guide prevention and intervention strategies (Weed et al, 1990; Lippman et al, 1990). Such intermediate elements prior to the development of metastatic capable cancers also provide the opportunity to define the molecular biology of these elements. Studies of the development of pre-invasive breast disease have provided insight into different types of lesions with different implications for breast cancer risk and the process of carcinogenesis (See Figure 1). Pre-invasive breast disease is herewith defined to be any reproducibly defined condition which confers an elevated risk of breast cancer approaching double that of the general population (Komitowski et al, 1990). The specifically-defined atypical hyperplasias and lobular carcinoma *in situ* confer relative risks of four to ten times that of the general population. This risk is for carcinoma to develop anywhere in either breast (Page et al, 1985; Page et al, 1991). The statistical significance of these observations have regularly been  $<.0001$ . Thus, absolute risk figures of 10-20% likelihood of developing into invasive carcinoma in 10 to 15 years arise. DCIS is a very special element in this story because the magnitude of risk is as high as any other condition noted ( $P < .00005$ ), but remarkably, the developing invasive cancer is in the same site in the same breast. This local recurrence and evolution to invasiveness marks these lesions as determinate precursors of invasive breast cancer (Betsill et al, 1978; Page et al, 1982). These figures are for the type of DCIS which has become detected very commonly since the advent of mammography, the small and NCDCIS variety. It is likely that the comedo DCIS variety indicates a much greater risk, often presenting as larger lesions, and treated regularly by mastectomy in the past 50 years making follow-up studies impossible (Figure 1).

The precision of histopathologic diagnosis in this area as noted in Table I (shown in Figure 1) was most convincingly confirmed in a large, prospective study (London et al, 1991). There has also been a recent review of the reproducibility of the assignment of diagnosis by a panel of pathologists (Schnitt et al, 1992). The precision has been fostered by combining histologic pattern criteria with cytologic and extent of

lesion criteria. Classic surgical pathology criteria were predominantly derived from histologic pattern only. A further point of relevance to the importance of these histopathologically defined lesions of pre-malignancy in the breast is the relationship to familiality. A family history of breast cancer in a first degree relatives confers about a doubling of breast cancer risk. However, women with the atypical hyperplasias at biopsy and a family history of breast cancer are at 9-10 times the risk of developing invasive breast cancer as the general population (Dupont et al, 1985; Dupont et al, 1989).

Careful consideration of all of the above-mentioned epidemiologic data has led to the following model for progression from generalized pre-malignant lesions to determinant lesions to invasive cancer. Figure 2 shows this model for the induction and progression of pre-invasive breast disease based on study of the Vanderbilt cohort (Dupont et al, 1985) of more than 10,000 breast biopsies (follow-up rate 85%; median time of 17 years; 135 women developed breast cancer).

15

## EXAMPLE 2

### Identification of genes which are differentially expressed in DCIS

#### Construction of cDNA libraries from DCIS lesions

In order to study differential gene expression in DCIS, we collected cases of NCDCIS. The diagnosis of DCIS is made on histomorphologic grounds based on architectural, cytologic, and occasionally extent criteria. NCDCIS lacks comedo features and consists of microscopic intraductal lesions which fill and extend the duct, contain rigid internal architecture, and often have hyperchromatic and monomorphic nuclei.

Study of non-comedo DCIS for differential marker gene expression indicates the diagnostic utility of comparison of marker gene expression in these tissues. Although the morbidity and mortality of breast cancer clearly results from invasion and metastasis, the development of breast cancer is clearly significant in its early stages for two basic reasons:

- 30 1) The molecular changes will presumably be simpler in early lesions than in later lesions which may have acquired numerous mutations or "hits";

and

2) Successful prevention strategies may require attacking cancer before it develops the capacity to invade or metastasize.

5 Non-comedo DCIS is the earliest determinant lesion which recurs locally as invasive cancer. Although comedo DCIS may be technically easier to study because the tumors are larger, its aggressiveness and the presence of numerous genetic alterations (such as p53 and erbB2) suggest that it may have advanced beyond the earliest stages of carcinogenesis.

10 The commercial utility of a method for prevention of cancer is clear. In order to study differential gene expression in DCIS, breast tissue with extensive microscopic non-comedo DCIS was identified and banked in a frozen state. cDNA libraries were constructed from mRNA isolated from frozen sections of DCIS lesions. Tissue samples from patients with mammographic results consistent with DCIS were cryostat frozen and a definitive diagnosis was made by the histopathologic criteria which we have described (Jensen et al, Submitted for publication; Holt et al, In press).

15 Control mRNA was obtained from frozen tissue samples obtained from reduction mammoplasties and from cultured human breast epithelial cells. Because non-comedo DCIS is a microscopic lesion, we had to microlocalize regions of DCIS in biopsy samples. To accomplish this we prepared frozen sections in which we located regions of DCIS and then employed a 2 mm punch to obtain an abnormal tissue sample only from those regions that contained DCIS. This selective harvesting was accomplished by carefully aligning the frozen section slide with the frozen tissue block and identifying areas of interest. The harvest of the appropriate area was then confirmed with a repeat frozen section. A similar approach was used to isolate mRNA from 20 lobules of normal breast in samples collected from a reduction mammoplasty. Prior studies have shown that breast lobules are approximately 2.5 mm in diameter, thus the 2 mm punch provided a well-tailored excision. This microlocation and collection step, 25 in which abnormal tissue samples are collected from an isolatable tissue structure, was performed with extreme care and was absolutely crucial to the success of these studies. Contamination by normal breast epithelial cells or by breast stromal cells would clearly 30 negatively skew the differential screening approach. If the punch biopsy did not cleanly

excise DCIS without contamination by other cell types or tissues then the sample was not used for mRNA isolation (Jensen et al, Submitted for publication). Figure 3 contains color photos of DCIS (abnormal) tissue, before (upper left panel) and after excisional punch biopsy (upper right panel). The lower panels show tissue samples of normal breast tissue (lower left panel), and invasive breast cancer (lower right panel).

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Following microlocation punch harvesting of the frozen tissue, RNA was isolated, purified, and employed to construct cDNA libraries. RNA was isolated following mincing of tissue in 5.6M guanidinium isothiocyanate and 40% phenol, centrifugation to remove particulate matter, viscosity reduction by repeated aspiration through a 22 gauge needle, chloroform extraction and ethanol precipitation. In most samples there was particulate matter resistant to guanidinium-phenol extraction that was white in color and fibrous in appearance and was presumed to represent breast stroma. This stromal material was sparse in DCIS samples but abundant in samples obtained from normal breast tissue derived from reduction mammoplasties. The stromal material was minced with a tissuemizer, washed with PBS, treated with collagenase at 37°C for 30 minutes, sonicated, extracted with phenol/chloroform and ethanol precipitated. 200 ug of total RNA was obtained by pooling 20 punches from normal breast tissue (reduction mammoplasty samples) and 5-8 punches from DCIS lesions, presumably reflecting the greater cellularity of the DCIS samples. All libraries had greater than 50% inserts and contained between  $2 \times 10^6$  and  $7 \times 10^7$  phage recombinants with an average insert size varying between 500 and 1000 base pairs.

### EXAMPLE 3

#### Development of an extraction method which produces breast epithelial RNA

25 It was necessary that tissue samples not be contaminated by non-epithelial stromal cells. Such contamination would complicate efforts to compare gene expression between samples. In order to test the extent of stromal contamination of the mRNA samples, we determined the level of expression of collagen III mRNA by an RNase protection assay. RNase protection assays were employed in these and subsequent studies because it is a quantitative method and can be performed on small amounts of 30 unselected RNA. Collagen III mRNA was identified in the presumed stromal fraction

of the normal breast tissue and to a lesser extent in the microinvasive breast cancer sample, but no expression of collagen III was detected in the DCIS samples which were subsequently employed for cDNA library construction. Figure 4 compares expression in NL 2 and #10CA with other patient samples and NL1 to determine collagen III expression.

5 Expression of Collagen III mRNA in tissue mRNA samples was analyzed by RNase protection assay by methods we have reported previously (Holt, 1993). One  $\mu$ g of mRNA was hybridized with two labeled RNA probes: a T7 polymerase-generated probe for human glyceraldehyde phosphate dehydrogenase (GADP) which protects a 10 140 bp Sac I-Xba I fragment; and a T7 polymerase-generated probe which protects a 208 bp Hinc II-Pst I fragment from the 3' untranslated region of the human type III procollagen gene (Coll III) obtained by PCR subcloning of the published sequence (Ala-Kokko et al, 1991). RNA samples were labeled as follows: NL1 is RNA from cultured 15 human breast epithelial cells (Hammond et al, 1984), NL2 is RNA from normal breast tissue, NL3 is RNA derived from the fibrous stromal fraction of breast tissue as described (Jensen et al, Submitted for publication), NL4 is another sample from normal breast tissue. This is described in greater detail on page 30 of this patent application. 20 #12, #8, #4, #6, and #10 are from patient samples with DCIS. Sample #10CA is RNA obtained from the small focus of microinvasion shown in Figure 3. Con is a control sample using tRNA.

#### EXAMPLE 4

##### Screening of cDNA libraries

25 Following successful testing which demonstrated that stromal contamination was not a problem, cDNA libraries were constructed in lambda phage using polyA-selected mRNA from the following samples: cultured human breast epithelial cells, tissue from three reduction mammoplasty patients, tissue from three DCIS patients, and tissue from one DCIS patient (patient #10) that showed a small focus of invasion adjacent to an 30 area of DCIS. Multiple punches were needed to obtain sufficient RNA for polyA selection and library construction. Selective handling of tissue was accomplished.

5 Comparison of gene expression between samples was performed by either differential screening or a modification of differential display (Liang et al, 1992a; Liang et al, 1992b; Saiki et al, 1988; Melton et al, 1984). Plasmid DNA was prepared from the cDNA libraries following helper phage rescue and screened by two independent methods. Figure 5 below shows the results of differential display comparing cDNAs of several patient DCIS samples with cDNA obtained from normal breast epithelial cells and an early invasive cancer. Although few genes shown in this Figure are differentially expressed in the majority of samples with DCIS, the heterogeneity of gene expression in patient samples is seen.

10 The differential display method (Liang et al, 1992a and 1992b) allows simultaneous comparison of multiple tissue samples. Initial studies using this method (reverse transcriptase followed by PCR) were unsatisfactory because of unwanted amplification of contaminating DNA in tissue samples and the small size of many of the fragments identified by display. To circumvent some of these problems, we have attempted to combine the advantages of cDNA library screening with the advantages of differential display by:

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- 1) Constructing cDNA libraries from the tissue mRNA samples;
- 2) Performing differential display on the plasmid DNA prepared from the cDNA libraries;
- 3) Subcloning the fragments identified by differential display;
- 20 4) Using the subcloned fragment as a probe to clone the cDNA from the appropriate library.

#### Example 5

##### Identification of a gene (RibRed) which is differentially expressed in multiple NCDCIS cases

25 Employing these methods, 10 differentially expressed clones were identified and the seven that showed the greatest difference in expression between multiple samples were further characterized by DNA sequencing. Comparison of the sequenced clones with GenBank demonstrated that six of the clones are apparently unique sequences (although further DNA sequencing is necessary); but that one of the clones (here termed DCIS-1 and described in Sequence Listing No. 1) showed 90% homology to the

previously cloned hamster gene encoding the M2 subunit of ribonucleotide reductase (Pavloff et al, 1992; Huria et al, 1991; Huria et al, 1991). Although human M2 ribonucleotide reductase has been cloned previously, comparison of the hamster cDNA sequence with our clone and with the prior human clone indicates that DCIS-1 is homologous to an alternatively poly-adenylated form of the human ribonucleotide reductase which has not been cloned previously. Figure 6 shows a comparison of the sequence between DCIS-1 and the human and hamster genes.

Because of our concern that different patients may have differential gene expression which is idiosyncratic (or related to morphological differences in biopsy appearance) and not necessarily related to the induction or progression of DCIS, we simultaneously analyzed gene expression in multiple DCIS samples compared to multiple control samples. We constructed cDNA libraries from the following samples:

- 1) Cultured HMEC epithelial cells;
- 2) Reduction mammoplasty: 11 year old with virginal hyperplasia;
- 3) Reduction mammoplasty: 28 year old patient;
- 4) Reduction mammoplasty: 35 year old patient;
- 5) DCIS patient #12;
- 6) DCIS patient #8;
- 7) DCIS patient #10;
- 8) DCIS patient #10 from an area of invasive cancer adjacent to DCIS;

In addition to the samples we employed to construct cDNA libraries shown above, we also obtained frozen tissue samples from 7 more DCIS patients, 2 cellular fibroadenoma samples, and samples of "usual hyperplasia" and atypical hyperplasia.

Because the DCIS clones were identified by cloning methods which include selection and amplification, it was important to confirm by nuclease protection assays that the genes were differentially expressed in the original unselected, unamplified RNA samples (Figure 7).

This approach allowed identification of a human gene similar to the hamster RibRed gene (coding for the M2 subunit) and 7 other human genes as genes which are differentially expressed in a majority of cases of DCIS in human breast tissue. The table of differentially expressed genes lists the genes which have been identified as

5 differentially expressed genes in DCIS tissue samples as compared to that in normal tissue (Figure 9).

#### EXAMPLE 6

##### Methods for studying potential use of differentially expressed genes for diagnostic screening

10 One advantage of the differential display method is that it allows comparison of multiple tissue samples of pre-invasive or invasive breast cancer. For example, use of this method has successfully demonstrated that the M2 subunit ribonucleotide reductase gene is differentially expressed in 4 out of 5 pre-invasive breast cancer tissue samples. It is significant that the M2 subunit is involved in the regulation of the ribonucleotide reductase gene and is found to be over-expressed in abnormal tissue samples.

15 Identification of differentially expressed genes may lead to discovery of genes which are potentially useful for breast cancer screening. Of particular interest are genes whose expression is restricted to breast epithelial cells and whose gene products are secreted. Screening for secreted proteins is possible by using the known hydrophobic sequences which encode leader sequences as one primer for differential display. The identification of secreted proteins which are specific for early breast pre-malignancy (or even early invasive cancer) would provide an important tool for early 20 breast cancer screening programs. If a differentially expressed gene has not been cloned previously (or if details of its expression are unknown or uncertain) then nuclease protection assays or Northern blots can be performed on RNA prepared from tissue samples from a variety of tissues to determine if expression of this gene is restricted to breast. If necessary cDNA libraries prepared from other tissues can be 25 added to the differential display screen as a way to identify only those genes which are expressed in early breast cancer and, in addition, are only expressed in breast tissue.

30 Once differentially expressed genes have been initially characterized for expression in pre-malignant and malignant breast disease, antibodies to the protein products of potentially useful genes can be developed and employed for immunohistochemistry (Harlow et al, 1988). This will provide an additional test to determine whether the expression of this gene is restricted to the breast. Subsequently, these antibodies will

be used to detect the presence of this protein present in the blood of patients with pre-invasive and/or invasive cancer. By assaying for serum protein levels in the same patients who exhibited elevated expression of the gene in their tissue samples it will be possible to determine whether a gene product is being secreted into the blood.

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#### EXAMPLE 7

##### **Decreased expression of BRCA1 accelerates growth and is observed during breast cancer progression**

Breast cancer occurs in hereditary and sporadic forms. Recently the BRCA 1 gene has been cloned and shown to be mutated in kindreds with hereditary breast and ovarian cancer (Hall et al. 1990, Miki, Y. et al. 1994, Friedman et al. 1994, Castilla et al. 1994, Simard et al. 1994). Although 92% of families with two or more cases of early-onset breast cancer and two cases of ovarian cancer have germ-line mutations in BRCA 1 (Narod et al. in press), the gene has not been shown to be mutated in any truly sporadic case to date (Futreal et al. 1994). Despite the surprising paucity of somatically acquired mutations in sporadic breast cancer, it is still a likely tumor suppressor gene with a key role in breast epithelial cell biology. The BRCA 1 gene encodes a protein of 1863 amino acids with a predicted zinc finger domain observed in proteins which regulate gene transcription.

As an initial characterization of the regulation and function of the BRCA 1 gene, we analyzed and manipulated expression of BRCA 1 mRNA levels. The results taken together indicate that the BRCA 1 gene product is a negative regulator of mammary cell proliferation which is expressed at diminished levels in sporadic breast cancer.

##### **Expression of BRCA1 mRNA during breast cancer progression**

As described above, microscopy-directed cloning has been employed to compare gene expression in normal mammary epithelium, carcinoma in-situ, and invasive breast cancer. This method produces predominantly epithelial mRNA with minimal contamination from stromal elements and we used this approach to obtain mRNA from normal neoplastic tissues from patients without a family history of breast cancer. Expression of BRCA1 exon 24 in human breast tissue samples is shown in Fig. 1. The legend of Fig. 1 is as follows.

The following tissue samples were used for mRNA isolation: Normal tissue samples: NL1-cultured human breast epithelial cells, NL2- Histologically normal breast tissue from an 11 year old undergoing a reduction mammoplasty, NL4- histologically normal breast tissue from an 14 year old undergoing a reduction mammoplasty. 5 Carcinoma-in-situ samples are #6, #8, #10, #12, #23 (comedo type), #41, #55; and invasive cancer samples #10CA (invasive cancer from the same patient with carcinoma-in-situ), 36CA, 1CA. All of these tissue samples were obtained from patients who had no family history of hereditary breast cancer and RNA preparation was performed as described above.

10 PCR detection of BRCA1 exon 24 in cDNA libraries from the following tissue samples is described in Figure 10A. Lane 1: human genomic DNA, lane 2: NL1, lane 3: NL4, lane 4: #8, lane 5: #12, lane 6: #10, lane 7: #10CA, lane 8: #41, lane 9: #23, lane 10: 36CA, lane 11: lambda DNA. The arrow points to the expected 113 bp band.

15 Nuclease protection assays of microdissected mRNA from tissue samples are described in Fig. 10B. One ug of mRNA from each tissue sample was hybridized with 32P-labelled, T7 polymerase-generated RNA probes for BRCA1 and human glyceraldehyde-3-phosphate dehydrogenase (GAPD) which produce expected protected fragments of 113 and 140 respectively as indicated by the lines on the right. Data were quantitated by phosphorimaging. The hybridizing intensity of each BRCA1 band was normalized to its respective GAPD band. The normalized values of NL1, NL2, and 20 NL4 were intensity in each sample relative to 1. Sample 1 employs human leukocyte mRNA; Samples 2-4 are NL1, NL2, and NL4; Samples 5-9 are #6(2.8), 8(3.7), 10(2.8), 12 (5.9), and 55 (1.4); and 10-12 are #10CA (0.07), 36CA (0.13), and 1CA (0.2).

25 Fig. 10 shows that BRCA1 exon 24 mRNA is expressed at 5-10 fold higher levels in normal mammary tissue than in invasive breast cancer samples. Initial studies showed detectable levels of BRCA1 cDNA in a cDNA library prepared from a tissue sample with preinvasive carcinoma-in-situ but not in normal breast cancer invasive breast cancer cDNA libraries (Figure 10A). Because this method is relatively insensitive we directly quantitated BRCA1 mRNA by nuclease protection assays in 30 RNA samples obtained by our microdissection method described above. These assays

indicate that expression of BRCA1 mRNA in micro-dissected normal mammary epithelial tissue (lanes 2-4, Figure 10B) is 5-15 fold higher than that in breast cancer (lanes 10-12, Figure 10B). The highest levels of BRCA1 are observed in samples from non-comedo ductal carcinoma-in-situ (lanes 5-9, Figure 10B), a premalignant breast lesion with a finite, but relatively low rate of progression to invasion (Betsill et al., 5 1978, Page, D.L. et al., 1982, Page and Dupont, 1990).

Because these studies suggested that invasive breast cancer exhibited lower mRNA levels than normal breast epithelial cells, we compared expression of paired samples of normal breast and invasive cancer from the same patient (Figure 11A; 10 compare lanes 2 and 3, 4 and 5, 6 and 7). The legend of Fig. 11 is as follows.

Nuclease protection assays of RNA obtained from paired samples of invasive breast cancer and histologically normal breast tissue are shown in Fig. 11A. Samples in lanes 2 and 3 (first patient), 4 and 5 (second patient), 6 and 7 (third patient) are from 15 invasive cancer and normal breast tissue respectively. Lane 1 is NL1 mRNA as described in legend to Fig. 10 and lane 8 is human leukocyte mRNA. Ratios of BRCA1/GAPD for each sample: lane 1: 25.9, lane 2: 1.8, lane 3: 7.6, lane 4: 2.0, lane 5: 12.4, lane 6: 0.7, lane 7: 6.0. The probes and methods are as described in Fig. 10 except the GAPD probe was of lower specific activity to improve quantitation.

Nuclease protection assays of RNA from a series of invasive breast cancer tissue samples (lanes 2-9 compared with NL1 (lane 1) and leukocyte mRNA (lane 10) are 20 shown in Fig. 11B. Ratios of BRCA1/GAPD for each sample: lane 1: 19.1, lane 2: 0.3, lane 3: 1.8, lane 4: 1.6, lane 5: 0.2, lane 6: 0.3, lane 7: 1.9, lane 8: 0, lane 9: 0.6.

Although the samples were paired in Fig. 11A, they were not microdissected 25 so this approach overestimates the relative expression level of invasive samples because they have a greater percentage of epithelial cells. RNA levels were four to eight fold higher in samples derived from normal breast than in samples derived from invasive breast cancer. We next analyzed expression levels in 8 non-hereditary invasive cancer samples (Figure 11B: lanes 2-7). Although these samples showed some variability in 30 expression level, all had lower levels of BRCA1 mRNA (determined by ratio of

BRCA1 to GAPD) than the primary breast epithelial cell line or the normal breast samples shown in Figure 11A.

#### Effects of BRCA1 gene inhibition on proliferative rate and gene expression

Having demonstrated that mRNA expression levels of BRCA1 are higher in normal mammary cells than in cancer cells, we used antisense methods to test the hypothesis that BRCA1 expression inhibits cell growth. Unmodified 18 base deoxyribonucleotide complementary to the BRCA1 translation initiation site were synthesized and added to cultures of primary mammary epithelial cells (Stampfer et al. 1980) or MCF-7 breast cancer cells (Soule and McGrath, 1980). Figure 12 is graph showing growth rate of human primary mammary epithelial cells (A), MCF-7 cells (B), retinal pigmented epithelial cells (C), cultured as described below. Points and bars represent the mean and the 95% confidence interval of triplicate counts of cells incubated with a single bolus of the indicated concentration of antisense or control sense deoxyribonucleotide.

The morphologic appearance of the cell lines was not noticeably changed by addition of antisense oligonucleotide, but the proliferative rate was faster. Incubation of cells with 40 uM anti-BRCA1 oligonucleotide produced accelerated growth of both normal (Figure 12A) and malignant mammary cells (Figure 12B), but did not affect the growth of human retinal pigmented epithelial cells (Figure 12C). An intermediate dose of anti-BRCA1 oligonucleotide produced a less pronounced but significant increase in cell growth rate. This was not a toxic effect of the oligonucleotide since a control "sense" oligomer with the same GC content did not increase the proliferation rate, and because an addition of a 10 fold excess of sense oligomer to the anti-BRCA1 oligomer reversed the growth activation.

In order to critically evaluate the function of BRCA1 gene inhibition on growth stimulation and cell cycle progression it was necessary to identify a gene whose expression is cell cycle regulated in human mammary cells. The gene encoding the M2 subunit of ribonucleotide reductase is amplified in conditions of nucleotide starvation (Hurta and Wright 1992) and as shown above, exhibits elevated levels of expression in premalignant breast disease. Because ribonucleotide reductase constitutes the rate limiting step in DNA synthesis, we reasoned that it might be cell cycle regulated in a

5 synchronous growth model such as MCF-7 cells which can be growth arrested by tamoxifen and then restimulated by estrogen (Aitken et al. 1985, Arteaga et al. 1989). MCF-7 cells were growth arrested by tamoxifen for 48 hours and then stimulated at time zero (0) with 1uM estradiol (+E) or control vehicle (-E). Inhibition of DNA synthesis by tamoxifen and induction of synthesis by estrogen were confirmed by nuclear labelling studies with tritiated thymidine.

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Fig. 13 panels A and B show that transcription of the ribonucleotide reductase M2 gene is cell cycle regulated, inhibited by tamoxifen, and induced by estrogen. Fig. 13A is a Northern blot of mRNA from synchronized MCF-7 cells. At the indicated time in hours, total cellular RNA was isolated and Northern blotting performed using the 1.6 Kb Eco RI fragment from our cloned human ribonucleotide reductase cDNA described above. Two mRNA species of 1.6 and 3.4 Kb are observed in these studies.

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Fig. 13B shows nuclear runon studies of synchronized MCF-7 cells were performed by our published methods (Holt et al 1988) employing the 1.6 Kb fragment of ribonucleotide reductase described above (RR); the 1.8 Kb fragment of Topoisomerase II (Topo) described in the Olsen et al. 1993); the 1.0 Kb cyclophilin gene (Thompson et al. 1994) used as a constitutive control; and 18S ribosomal RNA (Thompson et al. 1994). Con represents cells which were grown for 48 hours but not treated with tamoxifen.

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Antisense inhibition is a useful strategy for studying gene expression which is dependent on expression of the antisense target gene (Robinson-Benion and Holt, in press, 1995), e.g. genes whose expression is directly or indirectly dependent on BRCA1 levels. Fig. 14 demonstrates that antisense inhibition of BRCA1 results in a corresponding increased expression of M2 ribonucleotide reductase mRNA. A nuclease protection assay of mRNA derived from primary mammary epithelial cells (lanes 1-4, 9-10) or MCF-7 cells (lanes 5-8, 11-12) cultured for 4 days with antisense or control oligonucleotide was performed under the following conditions: no oligonucleotide (lanes 1 and 5); 40uM antiBRCA1 (lanes 2,6,10,12); 4uM antiBRCA1 (lanes 3 and 7); 40uM sense control (lanes 4,8,9,11). Probes for BRCA1 and GAPD are as described for

Figure 10, and the ribonucleotide reductase M2 probe (RR) detects the 200 bp probe is described above.

Ribonucleotide reductase mRNA levels are highest in samples treated with 40 uM anti-BRCA1 oligonucleotide for both primary mammary epithelial cells and for MCF-7 cells (Fig. 14). Antisense inhibition of BRCA1 results in a 70-90% inhibition of mRNA levels in anti-BRCA1 treated cells compared with cells treated with the "sense" control oligonucleotide (compare lanes 9 and 10, Fig. 14). Note that MCF-7 cells have lower levels of BRCA1 than the normal mammary epithelial cells (compare lanes 1 and 5, Fig. 14) anti-BRCA 1 since the antisense inhibition may drop BRCA1 levels below a critical threshold which normally functions to inhibit growth.

#### Methodology

**Tissue samples.** Freshly obtained breast biopsy or reduction mammoplasty specimens were frozen and then RNA was obtained following the microdissection method described above. Lesions were selected which were microlocalized and homogenous so that pure lesions could be obtained by 2 mm punches. Samples which had admixed normal epithelial, carcinoma-in-situ, or invasive cancer were not used for this study. Family history was obtained by chart review and/or interview to exclude familial breast cancer cases.

**Nuclease Protection Assays.** PCR primers were derived from BRCA1 sequence in GenBank (Accession number U14680); forward 5' CAATTGGGCAGATGTGT 3' and reverse 5' CTGGGGATCTGGGTATCA 3' which amplify a 113 bp region from exon 24, corresponding to bases 5587 to 5699 of the human BRCA1. This region was selected because this exon has not been reported to be differentially spliced unlike more 5' exons. The BRCA1 probe was cloned by subcloning this 113 bp band from normal human genomic DNA into PCRscriptSK and screening for correct orientation. One ug of mRNA from each tissue sample was hybridized with 32P-labelled, T7 polymerase-generated RNA probes for BRCA1 and human glyceraldehyde-3-phosphate dehydrogenase (GADP) which would produce expected protected fragments of 113 and 140 respectively. The construction and use of the GADP probe for RNA standardization has been described above. The probe for

ribonucleotide reductase M2 mRNA is the same as above and detects a 200 bp protected fragment.

20 Our results indicate that the BRCA1 gene is expressed at higher levels in normal  
mammary cells than in breast cancer cells and that diminished expression of BRCA1  
increased the proliferative rate of breast cells. This correlates well with the recent  
finding that patients with BRCA1 gene-linked hereditary breast cancer have tumors that  
grow more rapidly than comparable sporadic tumors (Marcus, J. et al. 1994). The  
decreased mRNA levels which were observed in sporadic breast cancers are not a  
consequence of differential splicing of the gene since the RNAs were quantitated with  
probes from the 3' end of the mRNA which is not a region where differential splicing  
is reported to occur (Miki, Y. et al 1994). Invasive sporadic cancers have BRCA1  
mRNA levels which vary from 0 (in one case) to 20% of the levels observed in normal  
30 human mammary epithelium.

Examples 8 and 9 describe applications of the discovery of the function of the BRCA1 gene. Example 8 describes a gene therapy method and example 9 describes a drug screening method. The discovery of the diminished expression of the BRCA1 mRNA in breast cancer using the microdissection techniques of this invention provides an important scientific basis for these examples.

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#### Example 8

##### Gene Therapy method based on determination of the function of the BRCA1 Gene

Viral vectors containing a DNA sequence that codes for a protein having an amino acid sequence as essentially set forth in SEQ ID NO:49 can be constructed using techniques that are well known in the art. This sequence includes the BRCA1 gene product. Viral vectors containing a DNA sequence essentially as set forth in SEQ ID NO:47 (the BRCA1 gene) can be also constructed using techniques that are well known in the art. Retroviral vectors, adenoviral vectors, or adeno-associated viral vectors are all useful methods for delivering genes into breast cancer cells. An excellent candidate for use in breast cancer gene therapy is a Moloney-based retroviral vector with a breast selective MMTV promoter which we have reported previously (Wong et al). The viral vector is constructed by cloning the DNA sequence essentially as set forth in SEQ ID:47 into a retroviral vector such as a breast selective vector. Most preferably, the full-length (coding region) cDNA for BRCA1 is cloned into the retroviral vector. The retroviral vector would then be transfected into virus producing cells in the following manner: Viruses are prepared by transfecting PA317 cells with retroviral vector DNAs which were purified as described in Wong et al. Following transfection, the PA317 cells are split and then treated with G418 until individual clones can be identified and expanded. Each clone is then screened for its titer by analyzing its ability to transfer G418 resistance (since the retroviral vector contains a Neomycin resistance gene). The clones which have the highest titer are then frozen in numerous aliquots and tested for sterility, presence of replication-competent retrovirus, and presence of mycoplasm. The methods generally employed for construction and production of retroviral vectors have been described in Muller, 1990.

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Once high titer viral vector producing clones have been identified, then patients with breast cancer can be treated by the following protocol: Viral vector expressing

BRCA1 is infused into either solid tumors or infused into malignant effusions as a means for altering the growth of the tumor (since it is shown above that the BRCA1 gene product decreases the growth rate of breast cancer cells). Because viral vectors can efficiently transduce a high percentage of cancer cells, the tumors would be growth inhibited.

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#### Example 9

#### Method of Screening Compounds Capable of Activating Promoter Region of the BRCA1 Gene

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The discovery of the function of the BRCA1 gene provides a clear utility in that induction of expression of the gene and the resulting increase in level of protein encoded by the gene in the breast cancer cell should slow the proliferation of the breast cancer cells. Induction of expression of the gene can be caused by administering a compound to a patient that stimulates the regulatory regions of this gene, such as the promoter.

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A method for screening compounds that activate the promoter of the BRCA1 gene is designed in the following way. A promoter sequence is a DNA segment that upregulates the expression of a gene. A sequence essentially as set forth in SEQ ID NO:48 can be ligated into a suitable vector, such as a plasmid, that contains a reporter gene using standard recombinant DNA techniques of restriction enzyme digests, ligation of fragment into vector, and transformation of bacteria. SEQ ID NO:48 includes the promoter sequence of the BRCA1 gene. A reporter gene is a gene that produces a readily detectable product. Examples of appropriate reporter genes which could be employed for this purpose include Beta-galactosidase or the chloramphenicol acetyltransferase gene.

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The BRCA1 promoter/reporter gene combination can then be cloned into an expression vector or viral vector by standard recombinant DNA methods. Breast cancer cells can then be transfected with the expression vector containing the BRCA1 promoter/reporter gene using standard transfection methods which we have reported previously (Holt et al. PNAS 1986). A stable transformant with appropriate low level expression (breast cancer cells have low level BRCA1 expression as shown above) will be identified and then characterized to demonstrate proper DNA integration and

expression. Methods of establishing and characterizing stable transformants have been described (Holt, MCB, 1994). Once an appropriate stable transformant cell line is identified, then we can plate the cell line in a manner than permits screening of hundreds or thousands of drugs or biological agents (for example in multiple 96 well microtiter plates). Level of expression of the reporter gene can be quantitated and agents which activate expression are thus identified. A positive result (i.e. induction of the promoter region) results in increased levels of the reporter gene resulting in either an increase in color (Beta-galactosidase assay) or specific radioactivity (Chloramphenicol acetyltransferase activity) through a reaction between the protein encoded by the reporter gene and a compound in the reaction medium. The compound produced by the reaction between the reporter gene protein and the compound in the reaction medium is the cause of the increase in color or specific radioactivity. These compounds can be called indicator compounds in that their presence indicates that the drug or biological agent activated the BRCA1 promoter. Methods for standardizing and performing Beta-galactosidase or chloramphenicol acetyltransferase assays have been reported (Holt et. al. MCB 1994). This method would be useful for initial screening of agents which increase BRCA1 expression. These agents could then be tested in more rigorous assays of breast cancer growth such as nude mouse tumor assays (Arteaga et al). This approach allows mass screening of large numbers of agents, sparing more rigorous animal tests for only promising compounds which score in the reporter gene assay described herein.

Thus, although there have been described particular embodiments of the present invention of a new and useful "Method for Detection and Treatment of Breast Cancer", it is not intended that such embodiments be construed as limitations upon the scope of this invention except as set forth in the following claims. It will be apparent to those skilled in the art that many changes and modifications may be made without departing from the invention in its broader aspects. For example, the above described techniques may be used in the diagnosis of other diseases and detection of differential genetic expression from microscopically-directed tissue samples of pathologic tissue. The production of a cDNA library produced as a result of the differential expression of genes in pathologic tissue in comparison to normal tissue provides the opportunity for

further adiagnostic capabilities. Further, although there have been described certain experimental conditions used in the preferred embodiment, it is not intended that such conditions be construed as limitations upon the scope of this invention except as set forth in the claims.

5 The following references are included to provide details of scientific technology herein incorporated by reference to the extent that they provide additional information for the purposes of indicating the background of the invention or illustrating the state of the art.

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## REFERENCES

Abendroth, C. S., H. H. Wang, et al. 1991. Comparative features of carcinoma *in situ* and atypical ductal hyperplasia of the breast on fine-needle aspiration biopsy specimens. *Am J Clin Pathol* 96(5): 654-659.

15 Aitken, S.C. & Lippman, M.E. Effect of estrogens and antiestrogens on growth-regulatory enzymes in human breast cancer cells in tissue culture. *Cancer Res.* 45, 1611-1620 (1985).

Ala-Kokko, L., S. Kontusaari, C. T. Baldwin, H. Kuivaniemi, D. J. Prockop. 1991. Structure of cDNA clones coding for the entire preproalpha I (III) chain of human type III procollagen. *Biochemical Journal* 260, 509-516.

20 Allred, D. C., G. M. Clark, et al. 1992. Overexpression of HER-2/neu and its relationship with other prognostic factors change during the progression of *in situ* to invasive breast cancer. *Hum Pathol* 23(9): 974-979.

25 Arteaga, C.L. et al. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. *J. Clin. Invest.* 84, 1418-1423 (1989).

Betsill, WL, Jr., PP Rosen, PH Lieberman, and GF Robbins. 1978. Intraductal carcinoma. Long term follow-up after biopsy only. *J.A.M.A.* 239:1863-1867.

30 Böcker, W., B. Bier, et al. 1992. An immunohistochemical study of the breast using antibodies to basal and luminal keratins, alpha-smooth muscle actin, vimentin, collagen IV and laminin. Part II: Epitheliosis and ductal carcinoma *in situ*. *Virchows Arch A Pathol Anat Hispathol* 421: 323-330.

Callahan R, CS Cropp, GR Merlo, DS Liscia, APM Cappa, and R. Lidereau. 1992. Somatic mutations and human breast cancer. *Cancer* 69:1582-86.

Carter D, RRL Smith. 1977. Carcinoma *in situ* of the breast. *Cancer* 40:1189-1193.

5 Castilla, L.C. et al. Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. *Nature Genet* 8, 387-391 (1994).

Cheickh MB, P Rouanet, G Louason, P Jeanteur, and C Theillet. 1992. An attempt to define sets of cooperating genetic alterations in human breast cancer. *Int. J. Cancer* 51:542-547.

10 Chen L-C, W Kurisu, B-M Ljung, ES Goldman, Dmorre II, and HS Smith. 1992. Heterogeneity for allelic loss in human breast cancer. *JNCI* 84:506-510.

Dupont, WD, Parl FF, Hartmann WH, Brinton LA, Winfield AC, Worrell JA, Schuyler PA, Plummer WD: Breast cancer risk associated with proliferative breast disease and atypical hyperplasia. *Cancer* 71:1258-1265, 1993.

15 Dupont WD, and DL Page. 1985. Risk factors for breast cancer in women with proliferative breast disease. *N. Engl. J. Med.* 312:146-151.

Dupont WD, DL Page, LW Rogers, and FF Parl. 1989. Influence of exogenous estrogens, proliferative breast disease, and other variables on breast cancer risk. *Cancer* 63:948-957.

20 Fentiman, I. S. 1992. Ductal carcinoma *in situ*. *BMJ* 304: 1261-1262.

Fisher, E. R. and R. Siderit .1992. Value of cytometric analysis for distinction of intraductal carcinoma of the breast. *Breast Cancer Res Treat* 21: 165-172.

Friedman, L.S. et al. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. *Nature Genet* 8, 399-404 (1994).

25 Futreal, A. et al. BRCA1 mutations in primary breast and ovarian carcinomas. *Science* 266, 120-122 (1994).

Hall, J.M. et al. Linkage of early-onset breast cancer to chromosome 17q21. *Science* 250, 1684-1689 (1990).

Hammond SL, RG Ham, and MR Stampfer. 1984. Serum-free growth of human mammary epithelial cells: rapid clonal growth in defined medium and extended serial passage with pituitary extract. *Proc. Natl. Acad. Sci. USA* 81:5435-5439.

5 Harlow E, D Lane. 1988. *Antibodies. A Laboratory Manual.* Cold Spring Harbor Laboratory Press.

Holt JT, RA Jensen, and DL Page. *Histopathology: Old Principles and new methods.* 1994. *Cancer Surveys.* In Press.

Holt, JT. Antisense rescue defines specialized and generalized functional domains for c-fos protein. 1993. *Molecular and Cellular Biology* 13, 3821-3830.

10 Holt, J.T., Redner, R.L., and Nienhuis, A.W. An oligomer complementary to c-myc mRNA inhibits proliferation of H-60 promyelocytic cells and induces differentiation. *Mol. Cell. Biol* 8, 963-973 (1988).

15 Hurta, RAR, J. A. Wright. 1992. Alteration in the activity and regulation of mammalian ribonucleotide reductase by a DNA damaging agent. *Journal of Biological Chemistry* 267, 7066-7071.

Hurta, RAR, S. K. Samuel, A. H. Greenberg, J. A. Wright. 1991. Early induction of ribonucleotide reductase gene expression by TGF-beta1 in malignant Ras transformed cell lines. *Journal of Biological Chemistry* 266, 24097-24100.

20 Jensen, RA, DL Page, and JT Holt. Identification of genes expressed in pre-malignant breast disease by microscopically-directed cloning. *Proc. Natn. Acad. Sci. U.S.A.* 91, 9257-9261 (1994).

Killeen, J. L. and H. Namiki .1991. DNA analysis of ductal carcinoma in situ of the breast: A comparison with histologic features. *Cancer* 68: 2602-2607.

25 Komitowski D, and C. Janson. 1990. Quantitative features of chromatin structure in the prognosis of breast cancer. *Cancer* 65:2725-2730.

Lawrence, G. 1991. Evaluation of treatment options for ductal carcinoma in situ of the breast. *Arch Surg* 126: 1541-1541.

30 Lennington, WJ, RA Jensen, LW Dalton, and DL Page. Ductal carcinoma in situ of the breast. *Cancer* 73: 118-124, 1994.

Liang P, and AB Pardee. 1992. Differential display of eukaryotic mRNA by means of the polymerase chain reaction. *Science* 257:967-971.

5 Liang P, L Averboukh, K Keyomarsi, R Sager, and AB Pardee. 1992. Differential display and cloning of messenger RNAs from human breast cancers versus mammary epithelial cells. *Cancer* 52:6966-6968.

Lippman SM, JS Lee, R Lotan, W Hittelman, MJ Wargovich, and WK Hong. 1990. Biomarkers as intermediate endpoints in chemoprevention trials. *JNCI* 82:555-560.

10 London SJ, JL Connolly, SJ Schnitt, and GA Colditz. 1991. A prospective study of benign breast disease and risk of breast cancer. *JAMA* 267:941-944.

Maguire, H. J., M. E. Hellman, et al. 1992. Expression of c-erbB-2 in situ and in adjacent invasive ductal adenocarcinomas of the female breast. *Pathobiology* 60: 117-121.

15 Marcus, J.N., Watson, P., Page, D.L. & Lynch, H.T. Pathology and heredity of breast cancer in younger women. *J. Nat. Cancer Inst. Monograph No.* 16, 23-34 (1994).

Melton, D.A., D.A. Krieg, M.R. Rebagliati, T. Flaniatis, K. Zinn, and M.R. Green. 1984. SP6 RNA polymerase. *Nucleic Acid Res.* 12:7035-7056.

20 Miki, Y, et al. A strong candidate gene for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266, 66-71 (1994).

Narod, S.A. et al. A heterogeneity analysis of 145 breast-ovarian cancer families. *Am. J. Human Genet.* (In Press)

25 Olsen, J.E., Winston, J.T., Whitlock, J.A., and Pledger, W.J. Cell cycle-dependent gene expression in V point-arrested Balb c 3T3 cells. *J. Cell. Phys.* 154, 333-342 (1993).

Ottesen, G. L., H. P. Graversen, et al. 1992. Ductal carcinoma in situ of the female breast: Short-term results of a prospective nationwide study. *Am J Surg Pathol* 16: 1183-1196.

30 Page DL, WD Dupont, LW Rogers, and M Landenberger. 1982. Intraductal carcinoma. Follow-up after biopsy only. *Cancer* 49:751-758.

Page DL, WD Dupont, LW Rogers, and MS Rados. 1985. Atypical hyperplastic lesions of the female breast. A long term follow-up study. *Cancer* 55:2698-2708.

Page, D.L. & Dupont, W.D. A typical hyperplastic lesions of the female breast. A long term follow up study. *Cancer* 66, 1326-1335 (1990).

5 Page DL, TE Kidd, WD Dupont, JF Simpson, and LW Rogers. 1991. Lobular neoplasia of the breast: higher risk for subsequent invasion predicted by more extensive disease. *Hum Pathol.* 22:1232-1239.

10 Page DL, R Vander Zwaag, LW Rogers, LT Williams, WE Alker, and WH Hartman. 1978. Relation between component parts of fibrocystic disease complex and breast cancer. *J.Natl. Cancer Inst.* 61:1055-1063.

15 Pavloff, N., D. Rivard, S. Masson, S. H. Shen, A. M. Mes-Masson. 1992. Sequence analysis of the large and small subunits of human ribonucleotide reductase. *DNA Sequence* 2, 227-234 .

Pierce, S. M., S. J. Schnitt, et al. 1992. What to do about mammographically detected ductal carcinoma in situ? *Cancer* 70: 2576-2578.

20 Poller, D. N., C. E. Hutchings, et al. 1992. p53 Protein expression in human breast carcinoma: Relationship to expression of epidermal growth factor receptor, c-erbB-2 protein overexpression, and oestrogen receptor. *Br J Cancer* 66: 583-588.

Poller, DN, EC Roberts, JA Bell, CW Elston, RW Blamey, and IO Ellis. p53 expression in mammary ductal carcinoma in situ: relationship to immunohistochemical expression of estrogen receptor and cerbB2 protein. *Human Pathology* 1993, 24:463-468.

25 Posner, M. C. and N. Wolmark 1992. Non-invasive breast carcinoma. *Breast Cancer Res Treat* 21: 155-164.

Radford DM, K Fair, AM Thompson, JH Ritter, M Holt, T Steinbrueck, W Wallace, Wells SA Jr, and HR Donis-Keller. Allelic loss on chromosome 17 in ductal carcinoma in situ of the breast. *Cancer Res* 1993, 53:2947-2950.

30 Saiki, R.K., D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, and H.A. Erlich. 1988. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.

Sambrook J, EF Fritsch, T Maniatis. 1989. Molecular Cloning Laboratory Manual, 2d Edition. Cold Spring Harbor Laboratory Press.

Sanchez LM, F Vizoso, I Diez-Itza, C Lopez-Otin. 1992. Cancer Res 32:95-100.

5 Schnitt, SJ, JL Connolly, FA Tavassoli, et al. 1992. Interobserver reproducibility in the diagnosis of ductal proliferative breast lesions using standardized criteria. Am.J. Surg. Pathol. 16:1133-1143.

10 Schwartz, G. F., G. C. Finkel, et al. 1992. Subclinical ductal carcinoma in situ of the breast: Treatment by local excision and surveillance alone. Cancer 70: 2468-2474.

15 Simard, J. et al. Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. *Nature Genet* 8, 392-398 (1994).

Simpson, T., R. C. Thirlby, et al. 1992. Surgical treatment of ductal carcinoma in situ of the breast: 10- to 20-year follow-up. Arch Surg 127: 468-472.

20 Solin, L. J., A. Recht, et al. 1991. Ten-year results of breast-conserving surgery and definitive irradiation for intraductal carcinoma (ductal carcinoma in situ) of the breast. Cancer 68: 2337-2344.

25 Soule, H.D. & McGrath, C.M. Estrogen responsive proliferation of clonal human breast carcinoma cells in athymic mice. *Cancer Letters* 10, 177-189 (1980).

Stampfer, M., Hallowes, R.C., & Hackett, A.J. Growth of normal human mammary cells in culture. *In Vitro* 16, 415-425 (1980).

Swain, S. M. 1992. Ductal carcinoma in situ. *Cancer Invest* 10: 443-454.

25 Tsuda H, K Iwaya, T Fukutomi, S Hirohashi. p53 mutations and c-erbB2 amplification in intraductal and invasive breast carcinomas of high histologic grade. *Jpn J Cancer Res* 1993, 84:394-401.

Thompson, M.E., Valentine, D.L., Strada, S.J., Wagner, J.A., and Scammell, J.G. Transcriptional regulation of secretogranin II and chromogranin B by cyclic AMP in a rat pheochromocytoma cell line. *Mol. Pharmacol.* 880-889 (1994).

30 Ward, B. A., C. F. McKhann, et al. 1992. Ten-year follow-up of breast carcinoma in situ in Connecticut. *Arch Surg* 12: 1392-1395.

Weed DL, P Greenwald, and JW Cullem. 1990. The future of cancer prevention and control. *Semin. Oncol.* 17:504-509.

Wellings SR, HM Jensen, and RG Marcum. 1975. An atlas of subgross pathology of the human breast with special reference to possible precancerous lesions. *J. Natl. Cancer Inst.* 55:231-273.

#### ADDITIONAL DESCRIPTION OF THE FIGURES

Figure 2: Model for premalignant conditions, highlighting magnitude of risk for progression to clinical malignancy. Terms from human breast neoplasia are used: no proliferative disease (No Pro), proliferative disease without atypia (PDWA), typical hyperplasia (AH), carcinoma in situ (CIS). As is proposal of tumor progression each stage is more likely to proceed to the next (dotted lines), but could also remain stable (horizontal lines, probably fairly frequent), or directly proceed to develop a clone of cells with malignant behavior (vertical lines, becoming more likely further to right.)

Figure 5: Differential display of cDNAs obtained from patient tissue samples and controls. Rescued cDNA library samples were used as templates for low stringency PCR with the primers 5'GATGAGTTCTGTGCCGTACAACTGG3' and 5'GGTTATCGAAATCAGCCACAGCGCC3'; 40 cycles were performed at conditions described above. Samples (See legend to Figure 4): Lane 1 - #12; Lanes 2 and 3: separate phage rescues of NL1 to show reproducibility of the assay; Lane 4 - #8; Lane 5 - #10; Lane 6 - #10CA; Lane 7 - control from the rescued phage vector without cDNA inserts. Arrows mark cDNAs which are overexpressed in DCIS versus normal. Arrowheads mark cDNAs which are differentially expressed in the invasive cancer (note this may reflect contamination from stromal cells). The bar marks a cDNA which is expressed in normal breast cells at higher levels than in DCIS or invasive cancer.

Figure 7: Expression of DCIS-1 mRNA in tissue mRNA samples analyzed by RNase protection assay. Probes: GADH probe and DCIS-1 clone probe which was generated by linearizing the rescued plasmid with *Pvu* II and should generate a 200 bp protected fragment. RNA samples were labeled as in the legend to Figure 4.

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(A) MEDIUM TYPE: Diskette, 3.50 inch, 800 kB storage  
(B) COMPUTER: IBM PC/XT/AT compatible  
(C) OPERATING SYSTEM: MS-DOS (version 5.0)  
(D) SOFTWARE: WordPerfect 5.1/WordPerfect Editor

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: U.S. 08/182,961  
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 264
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) ORIGINAL SOURCE

- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: ductal carcinoma in situ
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained from identification of differential gene expression

(viii) POSITION IN GENOME:

- (A) CHROMOSOME SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown

(ix) FEATURE:

- (A) NAME/KEY: DCIS-1
- (B) LOCATION: GenBank accession no. L2736
- (C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display
- (D) OTHER INFORMATION: gene encoding M2 subunit of humanribonucleotide reductase

(x) PUBLICATION INFORMATION: unpublished

(K) RELEVANT RESIDUES IN SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

TTGGAATTG GGTACGGGG CCCCCCACTG TGCGAATTC CTGCATGGG GGGATCCACT 60
AGTCAGAGC AGGCCGCCAC CGCTAGGACT CCAGCTTTG TTCGTTCCCT TTAGTGAGGG 120
TTAATTTCG AGCTGGCGT AATCATGGTC ATAGCTGTT CCTGTGTGAA ATTGTATCC 180
GCTCACAATT CCACACAAACAA TACGAGCCGG AAGCATAAAA GTGTAAAGCC TGGGGTGCCT 240
AATGAGTGAG CTAACTCACA TTAA 264

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) ORIGINAL SOURCE

ORGANISM: *Homo sapiens sapiens*

- (C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast

(G) CELL TYPE: ductal carcinoma in situ  
(H) CELL LINE: not derived from a cell line  
(I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:  
(A) LIBRARY: cDNA library derived from human  
(B) CLONE: obtained from identification of differential gene expression

(viii) POSITION IN GENOME:  
(A) CHROMOSOME SEGMENT: unknown  
(B) MAP POSITION: unknown  
(C) UNITS: unknown

(ix) FEATURE:  
(A) NAME/KEY: DCIS-2  
(B) LOCATION: GenBank accession no. L27637  
(C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display

(x) PUBLICATION INFORMATION: unpublished  
(K) RELEVANT RESIDUES IN SEQ ID NO: 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:  
TAGCCCGGTT ATCGAAATAG CCACAGCGCC TCTTCACAT CAGCAGTACG CGGCCAGTT 60  
GTACGGACAC GGA 73

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 46  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA  
(iii) HYPOTHETICAL: no  
(iv) ANTI-SENSE: no  
(v) ORIGINAL SOURCE  
(A) ORGANISM: *Homo sapiens sapiens*

- (C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: ductal carcinoma in situ
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained from identification of differential gene expression

(viii) POSITION IN GENOME:

- (A) CHROMOSOME SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown

(ix) FEATURE:

- (A) NAME/KEY: DCIS-3
- (B) LOCATION: L27638
- (C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display

(x) PUBLICATION INFORMATION: unpublished

(K) RELEVANT RESIDUES IN SEQ ID NO: 3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TGCCCGATGT GTGTCGTACA ACTGGCGCTG TGGCTGATT CGATAA 46

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) ORIGINAL SOURCE

(A) ORGANISM: *Homo sapiens sapiens*

(C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS

(D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: female breast

(G) CELL TYPE: ductal carcinoma in situ

(H) CELL LINE: not derived from a cell line

(I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: cDNA library derived from human

(B) CLONE: obtained from identification of differential gene expression

(viii) POSITION IN GENOME:

(A) CHROMOSOME SEGMENT: unknown

(B) MAP POSITION: unknown

(C) UNITS: unknown

(ix) FEATURE:

(A) NAME/KEY: DCIS-4

(B) LOCATION: L27640

(C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display

(x) PUBLICATION INFORMATION: unpublished

(K) RELEVANT RESIDUES IN SEQ ID NO: 4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TAGGCCATGA GTTCGTGTCC GTACAACTGG GGCGCTGTGG CTGATTCGA TANNNNNAGC 60  
ATCAGCCCCGA CG 72

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 84

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: cDNA to mRNA  
(iii) HYPOTHETICAL: no  
(iv) ANTI-SENSE: no  
(v) ORIGINAL SOURCE  
(A) ORGANISM: *Homo sapiens sapiens*  
(C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS  
(D) DEVELOPMENTAL STAGE: adult  
(F) TISSUE TYPE: female breast  
(G) CELL TYPE: ductal carcinoma *in situ*  
(H) CELL LINE: not derived from a cell line  
(I) ORGANELLE: no  
(vii) IMMEDIATE SOURCE:  
(A) LIBRARY: cDNA library derived from human  
(B) CLONE: obtained from identification of differential gene expression  
(viii) POSITION IN GENOME:  
(A) CHROMOSOME SEGMENT: unknown  
(B) MAP POSITION: unknown  
(C) UNITS: unknown  
(ix) FEATURE:  
(A) NAME/KEY: DCIS-5  
(B) LOCATION: L27641  
(C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display  
(x) PUBLICATION INFORMATION: unpublished  
(K) RELEVANT RESIDUES IN SEQ ID NO: 5  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:  
TAGCCCGGTT ATCGAAATCA GCCACAGCGC CTAACCTCTG CAGAAGCCTT TGACCATCAC 60  
CAGTTGTACG GACACGAAC CATC 84  
(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 99
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) ORIGINAL SOURCE

- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: ductal carcinoma in situ
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained from identification of differential gene expression

(viii) POSITION IN GENOME:

- (A) CHROMOSOME SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown

(ix) FEATURE:

- (A) NAME/KEY: DCIS-6
- (B) LOCATION: L27642
- (C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display

(x) PUBLICATION INFORMATION: unpublished

- (K) RELEVANT RESIDUES IN SEQ ID NO: 6

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GTGGTTCCG AAATTCCTGG GAAGGGGGGT GCTGGCGTGT GGAATTGTGG CGGCCCCCTGG 60  
TCTGCCGCGG CGTTTTTGT CTACATTCTGT CGTAGCTCG 99

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 88
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: no

## (iv) ANTI-SENSE: no

## (v) ORIGINAL SOURCE

- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE: sample of non-comedo DCIS
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: ductal carcinoma in situ
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained from identification of differential gene expression

## (viii) POSITION IN GENOME:

- (A) CHROMOSOME SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown

## (ix) FEATURE:

- (A) NAME/KEY: DCIS-7
- (B) LOCATION: L27643

(C) IDENTIFICATION METHOD: microscopically-directed sampling and differential display

(x) PUBLICATION INFORMATION: unpublished

(K) RELEVANT RESIDUES IN SEQ ID NO: 7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATCAGCCGCC GACATTGGG TACCCGGGCC CCCCCCTCCG TCGGAATTCC TCGAGCCGGG 60  
ATCCATAGGA TGTGGAGTTA GTTTGTT 88

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGCGACGGCC CGCGCTCTGC CAGGG 25

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

64

(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGCCCCCTGGG TTACCCCTCCCC CGCCCG 25

(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GGATGGCGTC CTGTAACCCG ACGCT 25

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ACTGGGCTGT CCTGGCGGTGG CGGGG 25

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTGAGAGGTA GCGCGCGGGA GGCTG 25

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GCCTGGCCGC GACACGGATT ACCGC 25

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TTAGGGCATG GTGGACCTGG AGACG 25

(2) INFORMATION FOR SEQ ID NO:15  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TGTGGTTACG TCAGCGAAGG TAATA 25

(2) INFORMATION FOR SEQ ID NO:16  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no

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(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

AGTCGCACGC ATGTCACGCT CCCGC 25

(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TATCCAAGCG GCAGGGCTACG AGGCC 25

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGCGCGCCCCG ACGGTCTGGT ATCTA 25

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CTCCCTCCCC GGACTCGGGG TTAGT 25

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ATGCGGGCGG CTCGGGCTG GTCGC 25

(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA
  - (A) DESCRIPTION: PCR primer
- (iii) HYPOTHETICAL: yes
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CGTGAAGCCT ATGCCCTCCC TCAAC 25

(2) INFORMATION FOR SEQ ID NO:22

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
  - (A) DESCRIPTION: PCR primer
- (iii) HYPOTHETICAL: yes
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: oligonucleotide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTGCCGTGGT AGCCCTTCAG CGATC 25

(2) INFORMATION FOR SEQ ID NO:23

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
  - (A) DESCRIPTION: PCR primer
- (iii) HYPOTHETICAL: yes
- (iv) ANTI-SENSE: no
- (v) FRAGMENT TYPE: oligonucleotide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GCGACACTAG GCTCCGGAG GAGGG 25

(2) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TGGGCCAGGC CTCCGGGCC 25

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CCGAACTGC GATAGCGTCC 25

(2) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

AGCGGACACC TGTTTCCCCGA GAGCC 25

(2) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

AACGGGTGGA CATCCGCCTG CGGCC 25

(2) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

TGAACCACGA TGTCAATCTGT CCCGA 25

(2) INFORMATION FOR SEQ ID NO:29

### (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

**(A) DESCRIPTION: PCR primer**

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) **FRAGMENT TYPE: oligonucleotide**

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

TCATCCCCGC CGAAAGACGC TCGCC 25

(2) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ATAGGCTGCG GCACGGCGCTG GGACT 25

(2) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GACCAGGTGC GCACGGAGCAT GTACA 25

(2) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ACCGTAGTCA TCGGCCTTCG CGCCC 25

(2) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GGCCCCCTAGC CCAGGGTGAAC GCCCCA 25

(2) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CCCAAGTGCTA CGGGGGGGCC CAAGC 25

(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CCTTCCTGGG TTACCTGCC TCGGG 25

(2) INFORMATION FOR SEQ ID NO:36  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

TCCGGACAGC AGCCACGCCA AGGGC 25

(2) INFORMATION FOR SEQ ID NO:37  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

ACGGCGCTGGT CCACCGAGGC CTGAT 25

(2) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CGATGCAAGG CCAGCAGCAC TCGAC 25

(2) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCCCGGAGC GGACCCACCGG ACGTG 25

(2) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

AGCGGGGAGG GATCGGGGGC CAAGC 25

(2) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GCTTGGTGTA GGCAGGCAGC TCTTA 25

(2) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CCACCCCTGT AGTGCGGGCT GCGAG 25

(2) INFORMATION FOR SEQ ID NO:43  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no  
(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GGAACCCGAC GCCCGTCCAG GGTTC 25

(2) INFORMATION FOR SEQ ID NO:44  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: DNA  
(A) DESCRIPTION: PCR primer  
(iii) HYPOTHETICAL: yes  
(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

TCCGGCAGCA AGGCCGGGAC GCTCC 25

(2) INFORMATION FOR SEQ ID NO:45

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GACGGGGGAC GGGCTAGGTG GCTTA 25

(2) INFORMATION FOR SEQ ID NO:46

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (A) DESCRIPTION: PCR primer

(iii) HYPOTHETICAL: yes

(iv) ANTI-SENSE: no

(v) FRAGMENT TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

CTTGTGCGC GCGGAGAGGG CTGCC 25

(2) INFORMATION FOR SEQ ID NO:47:

(i) **SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 5712
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(v) **ORIGINAL SOURCE**

- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE:
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: ductal carcinoma in situ, invasive breast cancer and normal breast tissue
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

(vii) **IMMEDIATE SOURCE:**

- (A) LIBRARY: cDNA library derived from human
- (B) CLONE: obtained using published sequence

(viii) **POSITION IN GENOME:**

- (A) CHROMOSOME SEGMENT: unknown
- (B) MAP POSITION: unknown
- (C) UNITS: unknown

(ix) **FEATURE:**

- (A) NAME/KEY: BRCA1
- (B) LOCATION: GenBank accession no. U14680
- (C) IDENTIFICATION METHOD: microscopically-directed sampling and nuclease protection assay
- (D) OTHER INFORMATION: gene encoding BRCA1 protein

## (x) PUBLICATION INFORMATION:

(A) AUTHORS: Miki, Y., et. al.

(B) TITLE: A strong candidate gene for the breast and ovarian cancer susceptibility gene BRCA1.

(C) JOURNAL: Science

(D) VOLUME: 266

(E) PAGES: 66-71

(F) DATE: 1994

(K) RELEVANT RESIDUES IN SEQ ID NO: 47

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

agctcgctga	gacttcctgg	accccgacc	aggctgtggg	gtttctcaga	taactgggcc	60
cctgcgtctc	gggggccttc	accctctgtct	ctgggttggaa	tccattggaa	cagaagaa	119
atg gat tta	tct gct ctt	cgc gtt gaa gaa	gtt caa aat	gtc att aat		167
Met Asp Leu	Ser Ala Leu	Arg Val Glu	Glu Val Gln	Asn Val Ile	Asn	
1	5	10	15			
gct atg cag	aaa atc tta	gag ttt ccc	atc ttt ctg	gag ttg atc	aag	215
Ala Met Gln	Lys Ile Leu	Glu Cys Pro	Ile Cys Leu	Glu Leu Ile	Lys	
20	25	30				
gaa cct gtc	tcc aca aag	tgt gac cac	ata ttt tgc	aaa ttt tgc	atg	263
Glu Pro Val	Ser Thr Lys	Cys Asp His	Ile Phe Cys	Lys Phe Cys	Met	
35	40	45				
ctg aaa ctt	ctc aac cag	aag aca	ggg cct tca	cag ttt ctt	tgt	311
Leu Lys	Leu Leu Asn	Gln Lys	Lys Gly	Pro Ser	Gln Cys Pro Leu Cys	
50	55	60				
aag aat	aat acc	aaa agg	atc cta	caa gaa	agt acg	359
Lys Asn Asp	Ile Thr Lys	Arg Ser	Leu Gln	Glu Ser	Thr Arg Phe Ser	
65	70	75	80			
caa ctt	gtt gaa	gag cta	ttt atc	att ttt	gct ttt	407
Gln Leu Val	Glu Leu Leu	Lys Ile	Ile Cys	Ala Phe	Gln Leu Asp	
85	90	95				
aca ggt	ttt gag	tat gca	aaac agc	tat aat	ttt gca	455
Thr Gly	Leu Glu	Tyr Ala	Asn Ser	Tyr Asn	Phe Ala Lys	
100	105	110				
aaac tct	cct gaa	cat cta	aaa gat	gaa gtt	tct atc	503
Asn Ser	Pro Glu	His Leu	Lys Asp	Glu Val	Ser Ile Ile Gln	
115	120	125				
ggc tac	aga aac	cgt gcc	aaa aca	atc cta	cag agt	551
Gly Tyr	Arg Asn	Arg Ala	Lys Arg	Leu Leu	Gln Ser	
130	135	140				
cct tcc	ttt cag	gaa aac	atc agt	atc gtc	caa ctc	599
Pro Ser	Leu Gln	Glu Thr	Ser Leu	Ser Val	Gln Leu	
145	150	155	160			

cact gtc aca act ctg egg aca aag cag cgg ata cca cct cca aag acg	647		
Thr Val Arg Thr Leu Arg Thr Lys Gln Arg Ile Gln Pro Gln Lys Thr			
165	170	175	
tct gtc tac att gaa ttg gga tct gat tct tct gaa gat acc gtt aat	695		
Ser Val Tyr Ile Glu Leu Gly Ser Asp Ser Ser Glu Asp Thr Val Asn			
180	185	190	
aag gca act tat tgc agt gtc gga gat cca gaa ttg tta caa atc acc	743		
Lys Ala Thr Tyr Cys Ser Val Gly Asp Gln Glu Leu Leu Gln Ile Thr			
195	200	205	
cct caa gga acc egg gat gaa atc aat ttc gat tct gca aaa aag gct	791		
Pro Gln Gly Thr Arg Asp Glu Ile Ser Leu Asp Ser Ala Lys Lys Ala			
210	215	220	
gct tgt gaa ttt tct gag acg gat gta aca aat act gaa cat cat caa	839		
Ala Cys Glu Phe Ser Glu Thr Asp Val Thr Asn Thr Glu His His Gln			
225	230	235	240
ccc aat aat aat gat ttg aac acc act gag aag cgt gca gct gag agg	887		
Pro Ser Asn Asn Asp Leu Asn Thr Thr Glu Lys Arg Ala Ala Glu Arg			
245	250	255	
cat cca gaa aag tat cag ggt aat aat gat tct gtt tca aac ttg cat gtc gag	935		
His Pro Glu Lys Tyr Gln Gly Ser Ser Val Ser Asn Leu His Val Glu			
260	265	270	
cca tgt ggc aca aat act cat gcc aac tca tta cag cat gag aac aac	983		
Pro Cys Gly Thr Asn Thr His Ala Ser Ser Leu Gln His Glu Asn Ser			
275	280	285	
agt tta tta ctc act aac gac aca aat gta gaa aag gct gaa ttc	1031		
Ser Leu Leu Leu Thr Lys Asp Arg Met Asn Val Glu Lys Ala Glu Phe			
290	295	300	
tgt aat aac aac aat cag cct ggc tta gca egg aac caa cat aac aca	1079		
Cys Asn Lys Ser Lys Gln Pro Gly Leu Ala Arg Ser Gln His Asn Arg			
305	310	315	320
tgg gct gga aat aag gaa aca aat tgt aat gat agg cgg act ccc aac aca	1127		
Trp Ala Gly Ser Lys Glu Thr Cys Asn Asp Arg Arg Thr Pro Ser Thr			
325	330	335	
gaa aac aag gta gat ctg aat gct gat ccc ctg tgt gag aca aac gaa	1175		
Glu Lys Lys Val Asp Leu Asn Ala Asp Pro Leu Cys Glu Arg Lys Glu			
340	345	350	
tgg aat aac cag aac ctg cca tgc tca gag aat cct aca gat aat gaa	1223		
Trp Asn Lys Gln Lys Leu Pro Cys Ser Glu Asn Pro Arg Asp Thr Glu			
355	360	365	
gtt gtt cct tgg aat aca cta aat aac aac aat gtt aat gag	1271		
Asp Val Pro Trp Ile Thr Leu Asn Ser Ser Ile Gln Lys Val Asn Glu			
370	375	380	
tgg ttt tcc aca aat gat gaa ctg tta ggt tct gat gac tca cat gat	1319		
Trp Phe Ser Arg Ser Asp Glu Leu Leu Gly Ser Asp Asp Ser His Asp			
385	390	395	400
ggg gag tct gaa tca aat gcc aac gta gct gat gta ttg gac gtt cta	1367		
Gly Glu Ser Glu Ser Asn Ala Lys Val Ala Asp Val Leu Asp Val Leu			
405	410	415	

aat gag gta gat gaa tat tct ggt tct tca gag aac ata gac tta ctg	1415
Asn Glu Val Asp Glu Tyr Ser Ser Glu Lys Ile Asp Leu Leu	
420 425 430	
gcc agt gat cct cat gag gct tta ata tgt aac aat gaa aca gtt cac	1463
Ala Ser Asp Pro His Glu Ala Leu Ile Cys Lys Ser Asp Arg Val His	
435 440 445	
tcc aac tca gta gag aat att gaa gac aac ata ttt ggg aac acc	1511
Ser Lys Ser Val Glu Ser Asp Ile Glu Asp Lys Ile Phe Gly Lys Thr	
450 455 460	
tat cgg aag aag gca aac ctc ccc aac tta aac cat gta act gaa aat	1559
Tyr Arg Lys Lys Ala Ser Leu Pro Asn Leu Ser His Val Thr Glu Asn	
465 470 475 480	
cta att ata gga gca ttt gtt act gag cca aac ata aac gag cgt	1607
Leu Ile Ile Gly Ala Phe Val Ser Glu Pro Gln Ile Ile Gln Glu Arg	
485 490 495	
ccc ctc aca aat aac tta aac cgt aac aag aca cct aca tca ggc ctt	1655
Pro Leu Thr Asn Lys Leu Lys Aeg Lys Arg Arg Pro Thr Ser Gly Leu	
500 505 510	
cet cct gag gat ttt atc aac aac gca aat ttg gca gtt caa aac act	1703
His Pro Glu Asp Phe Ile Lys Lys Ala Asp Leu Ala Val Gln Lys Thr	
515 520 525	
cct gaa atg ata bat cag gga act aac caa acg gag cag aat ggt caa	1751
Pro Glu Met Ile Asn Gln Gly Thr Asn Gln Thr Glu Gln Asn Gly Gln	
530 535 540	
gtg atg aat att act aat aat ggt cat gag aat aac aac aac ggt gat	1799
Val Met Asn Ile Thr Asn Ser Gly His Glu Asn Lys Thr Lys Gly Asp	
545 550 555	
tct att cag aat gag aac aat cct aac cca ata gaa tca ctc gaa aac	1847
Ser Ile Gln Asn Glu Lys Asn Pro Asn Pro Ile Glu Ser Leu Glu Lys	
560 565 570 575	
gaa tct gct ttc aac aac gct gaa cct ata aac aac aac aac aac	1895
Glu Ser Ala Phe Lys Thr Lys Ala Glu Pro Ile Ser Ser Ile Ser	
580 585 590	
aat atg gaa ctc gaa tta aat atc cac aat tca aac gca cct aac aac	1943
Asn Glu Leu Glu Leu Asn Ile Met His Asn Ser Lys Ala Pro Lys Lys	
595 600 605	
aat agg ctg agg aac aac tct tct acc aac aac gca cct aac aac aac	1991
Asn Arg Leu Arg Arg Lys Ser Ser Thr Arg His Ile His Ala Leu Glu	
610 615 620	
cta gta gtc aat aac aat cta aac cca cct aat tgt aat gca ttg caa	2039
Leu Val Val Ser Arg Asn Leu Ser Pro Pro Asn Cys Thr Glu Leu Gln	
625 630 635	
att gat aat aat tct aac aat gaa gag aac aac aac aac aac aac	2087
Ile Asp Ser Cys Ser Ser Glu Glu Ile Lys Lys Lys Tyr Asn	
640 645 650 655	
caa atg cca gtc agg cac aac aac cta caa ctc atg gaa ggt aac	2135
Gln Met Pro Val Arg His Ser Arg Asn Leu Gln Leu Met Glu Gly Lys	
660 665 670	

gaa cct gca act gga gcc aag aag aat gac aag cca aat gaa cag aca	2183
Glu Pro Ala Thr Gly Ala Lys Lys Ser Asn Lys Pro Asn Glu Gln Thr	
675 680 685	
agt aat aat cat gag aat gat act ttc cca gag ctg aat aca aat	2231
Ser Lys Arg His Asp Ser Asp Thr Phe Pro Glu Leu Lys Leu Thr Asn	
690 695 700	
gca cct ggt tct ttt act aat tgc aat aat aat aat aat aat aat aat	2279
Ala Pro Gly Ser Phe Thr Lys Cys Ser Asn Thr Ser Glu Leu Lys Glu	
705 710 715	
ttt gtc aat cct aat ctt cca aat gaa gaa aat gaa aat aat aat aat	2327
Phe Val Asn Pro Ser Leu Pro Arg Glu Glu Lys Glu Glu Lys Leu Glu	
720 725 730 735	
aca gtt aat gtt tct aat aat gct gaa gac ccc aat aat aat aat aat	2375
Thr Val Lys Val Ser Asn Asn Ala Glu Asp Pro Lys Asp Leu Met Leu	
740 745 750	
agt gga gaa aat gtt ttg caa act gaa aat aat tct gta gag aat aat aat	2423
Ser Gly Glu Arg Val Leu Gln Thr Glu Arg Ser Val Glu Ser Ser Ser	
755 760 765	
att tca ttg gta cct ggt act gat tat ggc act cag gaa aat aat aat	2471
Ile Ser Leu Val Pro Gly Thr Asp Tyr Gly Thr Gln Glu Ser Ile Ser	
770 775 780	
tta ctg gaa gtt aat act ctt ggg aat gca aat aca gaa cca aat aat	2519
Leu Leu Glu Val Ser Thr Leu Gly Lys Ala Lys Thr Glu Pro Asn Lys	
785 790 795	
tgt gtt aat cag ttt gca gca ttt gaa aat ccc aat gga cta att cat	2567
Cys Val Ser Gln Cys Ala Ala Phe Glu Asn Pro Lys Gly Leu Ile His	
800 805 810 815	
ggt ttt tcc aat	2615
Gly Cys Ser Lys Asp Asn Arg Asn Asp Thr Glu Gly Phe Lys Tyr Pro	
820 825 830	
ttt gga cat gaa gtt aat	2663
Leu Gly His Glu Val Asn His Ser Arg Glu Thr Ser Ile Glu Met Glu	
835 840 845	
gaa aat gaa ctt aat	2711
Glu Ser Glu Leu Asp Ala Gln Tyr Leu Gln Asn Thr Phe Lys Val Ser	
850 855 860	
aat cgc cag tca ttt gct ccc ttt tca aat cca gga aat gca gaa gag	2759
Lys Arg Gln Ser Phe Ala Pro Phe Ser Asn Pro Gly Asn Ala Glu Glu	
865 870 875	
gaa ttt gca aca ttc tct gcc ccc ttt ggg tcc tta aat gaa aat aat	2807
Glu Cys Ala Thr Phe Ser Ala His Ser Gly Ser Leu Lys Lys Gln Ser	
880 885 890 895	
cca aat gtc act ttt gaa ttt gaa aat aat aat aat aat aat aat aat	2855
Pro Lys Val Thr Phe Glu Cys Glu Gln Lys Glu Glu Asn Gln Gly Lys	
900 905 910	
aat gag ttt aat	2903
Asn Glu Ser Asn Ile Lys Pro Val Gln Thr Val Asn Ile Thr Ala Gly	
915 920 925	

ttt cct gtt ggt cag aaa gat aag cca gtt gat aat gcc aaa tgt	2951
Phe Pro Val Val Gly Gln Lys Asp Lys Pro Val Asp Asn Ala Lys Cys	
930 935 940	
agt atc aaa gga ggc tct egg ttt tgt cta tca tct cag ttc aea ggc	2999
Ser Ile Lys Gly Gly Ser Arg Phe Cys Leu Ser Ser Gln Phe Arg Gly	
945 950 955	
aac gaa act gga ctc att act cca aat aaa cat gga ctt tta cca aac	3047
Asn Glu Thr Gly Leu Ile Thr Pro Asn Lys His Gly Leu Leu Gln Asn	
960 965 970 975	
cca tat cgt ata cca cca ctt ttt ccc atc aag tca ttt gtt aaa act	3095
Pro Tyr Arg Ile Pro Pro Leu Phe Pro Ile Lys Ser Phe Val Lys Thr	
980 985 990	
aaa tgt aag aaa aat ctg cta gag gaa aac ttt gag gaa cat tca atg	3143
Lys Cys Lys Lys Asn Leu Leu Glu Glu Asn Phe Glu Glu His Ser Met	
995 1000 1005	
tca cct gaa aga gaa atg gga aat gag aac att cca agt aca gtg agc	3191
Ser Pro Glu Arg Glu Met Gly Asn Glu Asn Ile Pro Ser Thr Val Ser	
1010 1015 1020	
aca att aac cgt aat aac att aca gaa aat gtt ttt aaa gaa gcc aac	3239
Thr Ile Ser Arg Asn Asn Ile Arg Glu Asn Val Phe Lys Glu Ala Ser	
1025 1030 1035	
tca aac aat att aat gaa gta ggt tcc agt act aat gaa gtg ggc tcc	3287
Ser Ser Asn Ile Asn Glu Val Gly Ser Ser Thr Asn Glu Val Gly Ser	
1040 1045 1050 1055	
agt aat aat gaa ata ggt tcc agt aat gaa aac att cca gca gaa cta	3335
Ser Ile Asn Glu Ile Gly Ser Ser Asp Glu Asn Ile Gln Ala Glu Leu	
1060 1065 1070	
ggt aea aac aca ggg cca aaa ttg aat gct atg ctt aea tta ggg gtt	3383
Gly Arg Asn Arg Gly Pro Lys Leu Asn Ala Met Leu Arg Leu Gly Val	
1075 1080 1085	
ttg caa cct gag gtc tat aaa caa agt ctt cct gga agt aat tgt aag	3431
Leu Gln Pro Glu Val Tyr Lys Gln Ser Leu Pro Gly Ser Asn Cys Lys	
1090 1095 1100	
cat cct gaa ata aaa aag caa gaa tat gaa gaa gta gtt cag act gtt	3479
Mis Pro Glu Ile Lys Lys Gln Glu Tyr Glu Glu Val Val Gln Thr Val	
1105 1110 1115	
aat aca gat ttc tct cca tat ctg att tca gat aac tta gaa cag cct	3527
Asn Thr Asp Phe Ser Pro Tyr Leu Ile Ser Asp Asn Leu Glu Gln Pro	
1120 1125 1130 1135	
atg gga agt agt cat gca tct cag gtt tgt tct gag aca cct gat gac	3575
Met Gly Ser Ser His Ala Ser Gln Val Cys Ser Glu Thr Pro Asp Asp	
1140 1145 1150	
ctg tta gat gat ggt gaa ata aag gaa gat act agt ttt gct gaa aat	3623
Leu Leu Asp Asp Gly Glu Ile Lys Glu Asp Thr Ser Phe Ala Glu Asn	
1155 1160 1165	
gac aat aag gaa agt tct gct gtt ttt aac aaa aac gtc cag aaa gga	3671
Asp Ile Lys Glu Ser Ser Ala Val Phe Ser Lys Ser Val Gln Lys Gly	
1170 1175 1180	

gag ctt agc agg agt cct agc cct ttc acc cat aca cat ttg gct cag	3719
Glu Leu Ser Arg Ser Pro Ser Pro Phe Thr His Thr His Leu Ala Gln	
1185 1190 1195	
ggt tac cga aga ggg gcc aag aca tta gag tcc tca gaa gag aac tta	3767
Gly Tyr Arg Arg Gly Ala Lys Lys Leu Glu Ser Ser Glu Glu Asn Leu	
1200 1205 1210 1215	
tct agt gag gat gaa gag ctt ccc tgc ttc cca cac ttg tta ttt ggt	3815
Ser Ser Glu Asp Glu Glu Leu Pro Cys Phe Gln His Leu Leu Phe Gly	
1220 1225 1230	
aaa gta aac aat ata cct tct cag tct act agg cat agc acc gtt gtc	3863
Lys Val Asn Asn Ile Pro Ser Gln Ser Thr Arg His Ser Thr Val Ala	
1235 1240 1245	
acc gag tgt ctg tct aag aac aca gag gag aat tta tta tca ttg aag	3911
Thr Glu Cys Leu Ser Lys Asn Thr Glu Glu Asn Leu Ser Leu Lys	
1250 1255 1260	
aat agc tta aat gac tgc agt aac cag gta aat ttg gca aag gca tct	3959
Asn Ser Leu Asn Asp Cys Ser Asn Gln Val Ile Leu Ala Lys Ala Ser	
1265 1270 1275	
cag gaa cat cac ctt agt gag gaa aca aaa tgt tct gct agc ttg ttt	4007
Gln Glu His His Leu Ser Glu Glu Thr Lys Cys Ser Ala Ser Leu Phe	
1280 1285 1290 1295	
tct tca cag tgc agt gaa ttg gaa gac ttg aat gca aat aca aac acc	4055
Ser Ser Gln Cys Ser Glu Leu Glu Asp Leu Thr Ala Asn Thr Asn Thr	
1300 1305 1310	
cag gat cct ttg att ggt tct tcc aaa caa atg agg cat cag tct	4103
Gln Asp Pro Phe Leu Ile Gly Ser Ser Lys Gln Met Arg His Gln Ser	
1315 1320 1325	
gaa agc cag gga gtt ggt ctg agt gac aag gaa ttg gtt tca gat gat	4151
Glu Ser Gln Gly Val Gly Leu Ser Asp Lys Glu Leu Val Ser Asp Asp	
1330 1335 1340	
gaa gaa aca gga acg ggc ttg gaa gaa aat aat caa gaa gag caa agc	4199
Glu Glu Arg Gly Thr Gly Leu Glu Glu Asn Asn Gln Glu Glu Gln Ser	
1345 1350 1355	
atg gat tca aac tta ggt gaa gca gca tct ggg tgt gag agt gaa aca	4247
Met Asp Ser Asn Leu Gly Glu Ala Ala Ser Gly Cys Glu Ser Glu Thr	
1360 1365 1370 1375	
agc gtc tct gaa gac tgc tca ggg cta tcc tct cag agt gac att tta	4295
Ser Val Ser Glu Asp Cys Ser Gly Leu Ser Ser Gln Ser Asp Ile Leu	
1380 1385 1390	
acc act cag cag egg gat acc atg caa cat aac ctg ata aag ctc cag	4343
Thr Thr Gln Gln Arg Asp Thr Met Gln His Asn Leu Ile Lys Leu Gln	
1395 1400 1405	
cag gaa atg gct gaa cta gaa gct gtg tta gaa cag cat ggg agc cag	4391
Gln Glu Met Ala Glu Leu Glu Ala Val Leu Glu Gln His Gly Ser Gln	
1410 1415 1420	
cct tct aac agc tac cct tcc atc ata agt gac tct tct gcc ctt gag	4439
Pro Ser Asn Ser Tyr Pro Ser Ile Ile Ser Asp Ser Ser Ala Leu Glu	
1425 1430 1435	

gac	ctg	cga	aat	cca	gaa	caa	agc	aca	tca	gaa	aaa	gca	gtc	tta	act	4487
Asp	Leu	Arg	Asn	Pro	Glu	Gln	Ser	Thr	Ser	Glu	Lys	Val	Leu	Gln	Thr	
1440	1445														1455	
tca	cag	aaa	agt	agt	gaa	tac	cct	ata	agc	cag	aat	cca	gaa	ggc	ctt	4535
Ser	Gln	Lys	Ser	Ser	Glu	Tyr	Pro	Ile	Ser	Gln	Asn	Pro	Glu	Gly	Xaa	
1460	1465														1470	
tct	gct	gac	aag	ttt	gag	gtg	tct	gca	gat	agt	tct	acc	agt	aaa	aat	4583
Ser	Ala	Asp	Lys	Phe	Glu	Val	Ser	Ala	Asp	Ser	Ser	Thr	Ser	Lys	Asn	
1475	1480														1485	
aaa	gaa	cca	gga	gtg	gaa	agg	tca	tcc	cct	tct	aaa	tgc	cca	tca	tta	4631
Lys	Glu	Pro	Gly	Val	Glu	Arg	Ser	Ser	Pro	Ser	Lys	Cys	Pro	Ser	Leu	
1490	1495														1500	
gat	gat	agg	tgg	tac	atg	cac	agt	tgc	tct	ggg	agt	ctt	cag	aat	aga	4679
Asp	Asp	Arg	Trp	Tyr	Met	His	Ser	Cys	Ser	Gly	Ser	Leu	Gln	Asn	Arg	
1505	1510														1520	
aac	tac	cca	tct	caa	gag	gag	gtc	att	aag	gtt	gtt	gat	gtg	gag	gag	4727
Asn	Tyr	Pro	Pro	Gln	Glu	Glu	Leu	Ile	Lys	Val	Val	Asp	Val	Glu	Glu	
1525	1530														1535	
caa	cag	ctg	gaa	gag	tct	ggg	cca	cac	gat	ttg	acg	gaa	aca	tct	tac	4775
Gln	Gln	Leu	Glu	Glu	Ser	Gly	Pro	His	Asp	Leu	Thr	Glu	Thr	Ser	Tyr	
1540	1545														1550	
ttg	cca	agg	caa	gat	cta	gag	gga	acc	cct	tac	ctg	gaa	tct	gga	atc	4823
Leu	Pro	Arg	Gln	Asp	Leu	Glu	Gly	Thr	Pro	Tyr	Leu	Glu	Ser	Gly	Ile	
1555	1560														1565	
agc	ctc	tcc	tct	gat	gac	cct	gaa	tct	gat	cct	tct	gaa	gac	aga	gcc	4871
Ser	Leu	Phe	Ser	Asp	Asp	Pro	Glu	Ser	Asp	Pro	Ser	Glu	Asp	Arg	Ala	
1570	1575														1580	
cca	gag	tca	gct	cgt	gtt	ggc	aac	ata	cca	tct	tca	acc	tct	gca	ttg	4919
Pro	Glu	Ser	Ala	Arg	Val	Gly	Asn	Ile	Pro	Ser	Ser	Thr	Ser	Ala	Leu	
1585	1590														1600	
aaa	gtt	ccc	caa	ttg	aaa	gtt	gca	gaa	tct	gcc	cag	agt	cca	gtc	gtt	4967
Lys	Val	Pro	Gln	Leu	Lys	Val	Ala	Glu	Ser	Ala	Gln	Ser	Pro	Ala	Ala	
1605	1610														1615	
gct	cat	act	act	gat	act	gtt	ggg	tat	aat	gca	atg	gaa	gaa	agt	gtg	5015
Ala	His	Thr	Thr	Asp	Thr	Ala	Gly	Tyr	Asn	Ala	Met	Glu	Glu	Ser	Val	
1620	1625														1630	
agc	agg	agg	cca	gaa	ttg	aca	gct	tca	aca	gaa	egg	gtc	aac	aaa	5063	
Ser	Arg	Glu	Lys	Pro	Glu	Leu	Thr	Ala	Ser	Thr	Glu	Arg	Val	Asn	Lys	
1635	1640														1645	
aga	atg	tcc	atg	gtg	gtg	tct	ggc	ctg	acc	cca	gaa	gaa	ttt	atg	ctc	5111
Arg	Met	Ser	Met	Val	Val	Ser	Gly	Leu	Thr	Pro	Glu	Glu	Phe	Met	Leu	
1650	1655														1660	
gtg	tac	aag	ttt	gcc	aga	aaa	cac	cac	atc	act	aat	ctt	att			5159
Val	Tyr	Lys	Phe	Ala	Arg	Lys	Mis	Mis	Ile	Thr	Leu	Thr	Asn	Leu	Ile	
1665	1670														1675	
act	gaa	gag	act	act	cat	gtt	gtt	atg	aaa	aca	gat	gct	gag	ttt	gtg	5207
Thr	Glu	Glu	Thr	Thr	His	Val	Val	Met	Lys	Thr	Asp	Ala	Glu	Phe	Val	
1685	1690														1695	

tgt gaa cgg aca ctg aac tat ttt cta gga att gcg gga gga aac tgg	5255		
Cys Glu Arg Thr Leu Lys Tyr Phe Leu Gly Ile Ala Gly Gly Lys Trp			
1700	1705	1710	
gtt gtt aac tat ttc tgg gtg acc cag tct att aac gaa aca aac atg	5303		
Val Val Ser Tyr Phe Trp Val Thr Gln Ser Ile Lys Glu Arg Lys Met			
1715	1720	1725	
ctg aat gag cat gat ttt gaa gtc aca gga aat gtg gtc aat gga aca	5351		
Leu Asn Glu His Asp Phe Glu Val Arg Gly Asp Val Val Asn Gly Arg			
1730	1735	1740	
acc cac cca ggt cca aac cga gca aca gaa tcc cag gac aca aac atc	5399		
Asn His Gln Gly Pro Lys Arg Ala Arg Glu Ser Gln Asp Arg Lys Ile			
1745	1750	1755	1760
ttc aag ggg cta gaa atc tgt tgc tat ggg ccc ttc acc aac atg ccc	5447		
Phe Arg Gly Leu Glu Ile Cys Cys Tyr Gly Pro Phe Thr Asn Met Pro			
1765	1770	1775	
aca gat cca ctg gaa tgg atg gta cag ctg tgt ggt gct tct gtg gtg	5495		
Thr Asp Gln Leu Glu Trp Met Val Gln Leu Cys Gly Ala Ser Val Val			
1780	1785	1790	
aag gag ctt tca tca ttc acc ctt ggc aca ggt gtc cac cca att gtg	5543		
Lys Glu Leu Ser Ser Phe Thr Leu Gly Thr Gly Val His Pro Ile Val			
1795	1800	1805	
gtt gtg cag cca gat gcc tgg aca gag gac aat ggc ttc cat gca att	5591		
Val Val Gln Pro Asp Ala Trp Thr Glu Asp Asn Gly Phe His Ala Ile			
1810	1815	1820	
ggg cag atg tgt gag gca cct gtg acc cga gag tgg atg ttg gac	5639		
Gly Gln Met Cys Glu Ala Pro Val Val Thr Arg Glu Trp Val Leu Asp			
1825	1830	1835	1840
agt gta gca ctc tac cag tgc cag gag ctg gac acc tac ctg ata ccc	5687		
Ser Val Ala Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro			
1845	1850	1855	
cag atc ccc cac agc cac tac tgc	5712		
Gln Ile Pro His Ser His Tyr			
1860			

**(2) INFORMATION FOR SEQ ID NO:48:**

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH:** 1237
- (B) TYPE:** nucleic acid
- (C) STRANDEDNESS:** double
- (D) TOPOLOGY:** linear

**(ii) MOLECULE TYPE:** DNA regulatory sequence

**(iii) HYPOTHETICAL:** no

**(iv) ANTI-SENSE:** no

(v) ORIGINAL SOURCE

(A) ORGANISM: *Homo sapiens sapiens*

(C) INDIVIDUAL/ISOLATE:

(D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: female breast

(G) CELL TYPE: normal breast

(H) CELL LINE: not derived from a cell line

(I) ORGANELLE: no

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: cDNA library derived from human

(B) CLONE: obtained using published sequence

(viii) POSITION IN GENOME:

(A) CHROMOSOME SEGMENT: unknown

(B) MAP POSITION: unknown

(C) UNITS: unknown

(ix) FEATURE:

(A) NAME/KEY: BRCA1 promoter

(B) LOCATION:

(C) IDENTIFICATION METHOD: restriction enzyme digest

(D) OTHER INFORMATION: DNA sequence regulating gene encoding BRCA1 protein

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Brown et al.

(B) TITLE: Scientific Correspondence

(C) JOURNAL: Nature

(D) VOLUME: 372

(E) PAGES: 733

(F) DATE: 22/29 DECEMBER 1994

(K) RELEVANT RESIDUES IN SEQ ID NO: 48

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TTCCGGGACT CTACTACCTT TACCCAGACG AGAGGGTGA 60  
CCAGTTATCT GAGAAACCCC ACAGCCTGGT GCGGGGTCCA GGAAGTCTCA GCGAGCTCAC 120

GCGCGGCAGT CGCAGTTTA ATTATCTGT AATTCCCCGG CTTTCCGTI GCCACGGAAA 180  
 CCAAGGGGCT ACCGCTAACG AGCAGCCTCT CAGAATACGA AATCAAGGTG CAATCAGAGG 240  
 AAGGGAGGGA CAGAAAGAGC CAAGCGTCTC TCGGGGCTCT GGATTGGCCA CCCAGTCTGC 300  
 CCCCCGATGA CGTAAAAGGA AAGAGACGGA AGAGGAAGAA TTCTACCTGA GTTCCCGTA 360  
 AAGCGCCCGC CCTCTCGCT CTACGCTTCC AGTGGGGCT TATTACGTCA CAGTAATTGC 420  
 TGTACCAAGG TCAAGATCGC CACCTGAGGC CTGAATATCA GCGTAAGATA GTGTCCAAG 480  
 CAGTCTTAAG AAGAGGTCCC ATTACCCAC TCTTCCGCC CTAATGGAGT CCTCCAGTT 540  
 AGGTAATAA AAGGATTGTT GGGAGGTGGA GGGAAAGAAC TACTATTCC AACATCCATT 600  
 GCGGAACGAA AGGGCTTGGC CACACTGTTC CTTGGAAACT GTAGTCTTAT GGAGAGGAAC 660  
 ATCCAATACC AAAGCGGGCA CAATTCTCAC GGAAATCCAG TGGATAGATT GGAGACCTCC 720  
 GCGGGCTTAT ACATGTCAAC AGTAATATTG GTTGTGTTATC TTCTCTTATC TTGAGAGCAG 780  
 AGACTAGGCC AAAAAAGAT ATAGGAAGAC TACGATTCCC ATCCAGCCCC ACGAGTCTCG 840  
 GGCAAGTAGT CCTCTAAGGT CAGTGGCCTG CGGGGACGCA GTGGGCGCCG AATTGCTG 900  
 GGGAAAGGGGA AATCCCTCTC TGGTACACATC TGGCCACTCC TAGTCCGCC CCTCAGCATC 960  
 AATGTTTGTGTT ATTGTTGTTG GGGTTCAAGGT TGCTTCTGCC CGGCCCCATC GACGCAATCT 1020  
 CCACCAATCA ATGGCGTGGT CGTTTGAGG GACAAGTGGT GAGAGCCAAT CATCTGGCG 1080  
 AACACTCGGA GAAACAGGGG ACTAGTTACT GTCTTATCC GCCATGTTAG ATTCAACCCA 1140  
 CAGGGATAGC GCGAGAGGCC GTAGCGGAGC GTCTTGCAT TGGCCTCCGG CAGGCGCCCC 1200  
 CGGGGGCCGG GAAGCTGGTA AGGAAGCAGC TCCGGTT 1237

**(2) INFORMATION FOR SEQ ID NO:49:**

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 1863
- (B) TYPE: amino acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

**(ii) MOLECULE TYPE: protein**

**(iii) HYPOTHETICAL: no**

**(iv) ANTI-SENSE: no**

**(v) ORIGINAL SOURCE**

- (A) ORGANISM: Homo sapiens sapiens
- (C) INDIVIDUAL/ISOLATE:
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: female breast
- (G) CELL TYPE: normal breast tissue
- (H) CELL LINE: not derived from a cell line
- (I) ORGANELLE: no

**(ix) FEATURE:**

- (A) NAME/KEY: BRCA1 protein

2 (B) LOCATION: 1 to 1863  
(C) IDENTIFICATION METHOD: observation of mRNA and antisense inhibition of BRCA1 gene  
(D) OTHER INFORMATION: BRCA1 protein has a negative regulatory effect on growth of human mammary cells.

(x) PUBLICATION INFORMATION:

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Met Asp Leu Ser Ala Leu Arg Val Glu Glu Val Gln Asn Val Ile Asn  
1 5 10 15  
Ala Met Gln Lys Ile Leu Glu Cys Pro Ile Cys Leu Glu Leu Ile Lys  
20 25 30  
Glu Pro Val Ser Thr Lys Cys Asp His Ile Phe Cys Lys Phe Cys Met  
35 40 45  
Leu Lys Leu Leu Asn Gln Lys Lys Gly Pro Ser Gln Cys Pro Leu Cys  
50 55 60  
Lys Asn Asp Ile Thr Lys Arg Ser Leu Gln Glu Ser Thr Arg Phe Ser  
65 70 75 80  
Gln Leu Val Glu Glu Leu Leu Lys Ile Ile Cys Ala Phe Gln Leu Asp  
85 90 95  
Thr Gly Leu Glu Tyr Ala Asn Ser Tyr Asn Phe Ala Lys Glu Asn  
100 105 110  
Asn Ser Pro Glu His Leu Lys Asp Glu Val Ser Ile Ile Gln Ser Met  
115 120 125  
Gly Tyr Arg Asn Arg Ala Lys Arg Leu Leu Gln Ser Glu Pro Glu Asn  
130 135 140  
Pro Ser Leu Gln Glu Thr Ser Leu Ser Val Gln Leu Ser Asn Leu Gly  
145 150 155 160  
Thr Val Arg Thr Leu Arg Thr Lys Gln Arg Ile Gln Pro Gln Lys Thr  
165 170 175  
Ser Val Tyr Ile Glu Leu Gly Ser Asp Ser Ser Glu Asp Thr Val Asn  
180 185 190

Lys Ala Thr Tyr Cys Ser Val Gly Asp Gln Glu Leu Leu Gln Ile Thr  
 195 200 205  
 Pro Gln Gly Thr Arg Asp Glu Ile Ser Leu Asp Ser Ala Lys Lys Ala  
 210 215 220  
 Ala Cys Glu Phe Ser Glu Thr Asp Val Thr Asn Thr Glu His His Gln  
 225 230 235 240  
 Pro Ser Asn Asn Asp Leu Asn Thr Thr Glu Lys Arg Ala Ala Glu Arg  
 245 250 255  
 His Pro Glu Lys Tyr Gln Gly Ser Ser Val Ser Asn Leu His Val Glu  
 260 265 270  
 Pro Cys Gly Thr Asn Thr His Ala Ser Ser Leu Gln His Glu Asn Ser  
 275 280 285  
 Ser Leu Leu Leu Thr Lys Asp Arg Met Asn Val Glu Lys Ala Glu Phe  
 290 295 300  
 Cys Asn Lys Ser Lys Gln Pro Gly Leu Ala Arg Ser Gln His Asn Arg  
 305 310 315 320  
 Trp Ala Gly Ser Lys Glu Thr Cys Asn Asp Arg Arg Thr Pro Ser Thr  
 325 330 335  
 Glu Lys Lys Val Asp Leu Asn Ala Asp Pro Leu Cys Glu Arg Lys Glu  
 340 345 350  
 Trp Asn Lys Gln Lys Leu Pro Cys Ser Glu Asn Pro Arg Asp Thr Glu  
 355 360 365  
 Asp Val Pro Trp Ile Thr Leu Asn Ser Ser Ile Gln Lys Val Asn Glu  
 370 375 380  
 Trp Phe Ser Arg Ser Asp Glu Leu Leu Gly Ser Asp Asp Ser His Asp  
 385 390 395 400  
 Gly Glu Ser Glu Ser Asn Ala Lys Val Ala Asp Val Leu Asp Val Leu  
 405 410 415  
 Asn Glu Val Asp Glu Tyr Ser Gly Ser Ser Glu Lys Ile Asp Leu Leu  
 420 425 430  
 Ala Ser Asp Pro His Glu Ala Leu Ile Cys Lys Ser Asp Arg Val His  
 435 440 445  
 Ser Lys Ser Val Glu Ser Asp Ile Glu Asp Lys Ile Phe Gly Lys Thr  
 450 455 460  
 Tyr Arg Lys Lys Ala Ser Leu Pro Asn Leu Ser His Val Thr Glu Asn  
 465 470 475 480  
 Leu Ile Ile Gly Ala Phe Val Ser Glu Pro Gln Ile Ile Gln Glu Arg  
 485 490 495  
 Pro Leu Thr Asn Lys Leu Lys Arg Lys Arg Arg Pro Thr Ser Gly Leu  
 500 505 510  
 His Pro Glu Asp Phe Ile Lys Lys Ala Asp Leu Ala Val Gln Lys Thr  
 515 520 525  
 Pro Glu Met Ile Asn Gln Gly Thr Asn Gln Thr Glu Gln Asn Gly Gln  
 530 535 540  
 Val Met Asn Ile Thr Asn Ser Gly His Glu Asn Lys Thr Lys Gly Asp  
 545 550 555  
 Ser Ile Gln Asn Glu Lys Asn Pro Asn Pro Ile Glu Ser Leu Glu Lys  
 560 565 570 575

Glu Ser Ala Phe Lys Thr Lys Ala Glu Pro Ile Ser Ser Ser Ile Ser  
 580 585 590  
 Asn Glu Leu Glu Leu Asn Ile Met His Asn Ser Lys Ala Pro Lys Lys  
 595 600 605  
 Asn Arg Leu Arg Arg Lys Ser Ser Thr Arg His Ile His His Ala Leu Glu  
 610 615 620  
 Leu Val Val Ser Arg Asn Leu Ser Pro Pro Asn Cys Thr Glu Leu Gln  
 625 630 635  
 Ile Asp Ser Cys Ser Ser Ser Glu Glu Ile Lys Lys Lys Tyr Asn  
 640 645 650 655  
 Gln Met Pro Val Arg His Ser Arg Asn Leu Gln Leu Met Glu Gly Lys  
 660 665 670  
 Glu Pro Ala Thr Gly Ala Lys Lys Ser Asn Lys Pro Asn Glu Gln Thr  
 675 680 685  
 Ser Lys Arg His Asp Ser Asp Thr Phe Pro Glu Leu Lys Leu Thr Asn  
 690 695 700  
 Ala Pro Gly Ser Phe Thr Lys Cys Ser Asn Thr Ser Glu Leu Lys Glu  
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 Phe Val Asn Pro Ser Leu Pro Arg Glu Glu Lys Glu Glu Lys Leu Glu  
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 Thr Val Lys Val Ser Asn Asn Ala Glu Asp Pro Lys Asp Leu Met Leu  
 740 745 750  
 Ser Gly Glu Arg Val Leu Gln Thr Glu Arg Ser Val Glu Ser Ser Ser  
 755 760 765  
 Ile Ser Leu Val Pro Gly Thr Asp Tyr Gly Thr Gln Glu Ser Ile Ser  
 770 775 780  
 Leu Leu Glu Val Ser Thr Leu Gly Lys Ala Lys Thr Glu Pro Asn Lys  
 785 790 795  
 Cys Val Ser Gln Cys Ala Ala Phe Glu Asn Pro Lys Gly Leu Ile His  
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 Gly Cys Ser Lys Asp Asn Arg Asn Asp Thr Glu Gly Phe Lys Tyr Pro  
 820 825 830  
 Leu Gly His Glu Val Asn His Ser Arg Glu Thr Ser Ile Glu Met Glu  
 835 840 845  
 Glu Ser Glu Leu Asp Ala Gln Tyr Leu Gln Asn Thr Phe Lys Val Ser  
 850 855 860  
 Lys Arg Gln Ser Phe Ala Pro Phe Ser Asn Pro Gly Asn Ala Glu Glu  
 865 870 875  
 Glu Cys Ala Thr Phe Ser Ala His Ser Gly Ser Leu Lys Lys Gln Ser  
 880 885 890 895  
 Pro Lys Val Thr Phe Glu Cys Glu Gln Lys Glu Glu Asn Gln Gly Lys  
 900 905 910  
 Asn Glu Ser Asn Ile Lys Pro Val Gln Thr Val Asn Ile Thr Ala Gly  
 915 920 925  
 Phe Pro Val Val Gly Gln Lys Asp Lys Pro Val Asp Asn Ala Lys Cys  
 930 935 940  
 Ser Ile Lys Gly Gly Ser Arg Phe Cys Leu Ser Ser Gln Phe Arg Gly  
 945 950 955

Asn Glu Thr Gly Leu Ile Thr Pro Asn Lys His Gly Leu Leu Gln Asn  
 960 965 970 975  
 Pro Tyr Arg Ile Pro Pro Leu Phe Pro Ile Lys Ser Phe Val Lys Thr  
 980 985 990  
 Lys Cys Lys Lys Asn Leu Leu Glu Glu Asn Phe Glu Glu His Ser Met  
 995 1000 1005  
 Ser Pro Glu Arg Glu Met Gly Asn Glu Asn Ile Pro Ser Thr Val Ser  
 1010 1015 1020  
 Thr Ile Ser Arg Asn Asn Ile Arg Glu Asn Val Phe Lys Glu Ala Ser  
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 Ser Ser Asn Ile Asn Glu Val Gly Ser Ser Thr Asn Glu Val Gly Ser  
 1040 1045 1050 1055  
 Ser Ile Asn Glu Ile Gly Ser Ser Asp Glu Asn Ile Gln Ala Glu Leu  
 1060 1065 1070  
 Gly Arg Asn Arg Gly Pro Lys Leu Asn Ala Met Leu Arg Leu Gly Val  
 1075 1080 1085  
 Leu Gln Pro Glu Val Tyr Lys Gln Ser Leu Pro Gly Ser Asn Cys Lys  
 1090 1095 1100  
 His Pro Glu Ile Lys Lys Gln Glu Tyr Glu Glu Val Val Gln Thr Val  
 1105 1110 1115  
 Asn Thr Asp Phe Ser Pro Tyr Leu Ile Ser Asp Asn Leu Glu Gln Pro  
 1120 1125 1130 1135  
 Met Gly Ser Ser His Ala Ser Gln Val Cys Ser Glu Thr Pro Asp Asp  
 1140 1145 1150  
 Leu Leu Asp Asp Gly Glu Ile Lys Glu Asp Thr Ser Phe Ala Glu Asn  
 1155 1160 1165  
 Asp Ile Lys Glu Ser Ser Ala Val Phe Ser Lys Ser Val Gln Lys Gly  
 1170 1175 1180  
 Glu Leu Ser Arg Ser Pro Ser Pro Phe Thr His Thr His Leu Ala Gln  
 1185 1190 1195  
 Gly Tyr Arg Arg Gly Ala Lys Lys Leu Glu Ser Ser Glu Glu Asn Leu  
 1200 1205 1210 1215  
 Ser Ser Glu Asp Glu Glu Leu Pro Cys Phe Gln His Leu Leu Phe Gly  
 1220 1225 1230  
 Lys Val Asn Asn Ile Pro Ser Gln Ser Thr Arg His Ser Thr Val Ala  
 1235 1240 1245  
 Thr Glu Cys Leu Ser Lys Asn Thr Glu Glu Asn Leu Leu Ser Leu Lys  
 1250 1255 1260  
 Asn Ser Leu Asn Asp Cys Ser Asn Gln Val Ile Leu Ala Lys Als Ser  
 1265 1270 1275  
 Gln Glu His His Leu Ser Glu Glu Thr Lys Cys Ser Ala Ser Leu Phe  
 1280 1285 1290 1295  
 Ser Ser Gln Cys Ser Glu Leu Glu Asp Leu Thr Ala Asn Thr Asn Thr  
 1300 1305 1310  
 Gln Asp Pro Phe Leu Ile Gly Ser Ser Lys Gln Met Arg His Gln Ser  
 1315 1320 1325  
 Glu Ser Gln Gly Val Gly Leu Ser Asp Lys Glu Leu Val Ser Asp Asp  
 1330 1335 1340

Glu Glu Arg Gly Thr Gly Leu Glu Glu Asn Asn Gln Glu Glu Gln Ser  
 1345 1350 1355  
 Met Asp Ser Asn Leu Gly Glu Ala Ala Ser Gly Cys Glu Ser Glu Thr  
 1360 1365 1370 1375  
 Ser Val Ser Glu Asp Cys Ser Gly Leu Ser Ser Gln Ser Asp Ile Leu  
 1380 1385 1390  
 Thr Thr Gln Gln Arg Asp Thr Met Gln His Asn Leu Ile Lys Leu Gln  
 1395 1400 1405  
 Gln Glu Met Ala Glu Leu Glu Ala Val Leu Glu Gln His Gly Ser Gln  
 1410 1415 1420  
 Pro Ser Asn Ser Tyr Pro Ser Ile Ile Ser Asp Ser Ser Ala Leu Glu  
 1425 1430 1435  
 Asp Leu Arg Asn Pro Glu Gln Ser Thr Ser Glu Lys Val Leu Gln Thr  
 1440 1445 1450 1455  
 Ser Gln Lys Ser Ser Glu Tyr Pro Ile Ser Gln Asn Pro Glu Gly Xaa  
 1460 1465 1470  
 Ser Ala Asp Lys Phe Glu Val Ser Ala Asp Ser Ser Thr Ser Lys Asn  
 1475 1480 1485  
 Lys Glu Pro Gly Val Glu Arg Ser Ser Pro Ser Lys Cys Pro Ser Leu  
 1490 1495 1500  
 Asp Asp Arg Trp Tyr Met His Ser Cys Ser Gly Ser Leu Gln Asn Arg  
 1505 1510 1515 1520  
 Asn Tyr Pro Pro Gln Glu Glu Leu Ile Lys Val Val Asp Val Glu Glu  
 1525 1530 1535  
 Gln Gln Leu Glu Glu Ser Gly Pro His Asp Leu Thr Glu Thr Ser Tyr  
 1540 1545 1550  
 Leu Pro Arg Gln Asp Leu Glu Gly Thr Pro Tyr Leu Glu Ser Gly Ile  
 1555 1560 1565  
 Ser Leu Phe Ser Asp Asp Pro Glu Ser Asp Pro Ser Glu Asp Arg Ala  
 1570 1575 1580  
 Pro Glu Ser Ala Arg Val Gly Asn Ile Pro Ser Ser Thr Ser Ala Leu  
 1585 1590 1595 1600  
 Lys Val Pro Gln Leu Lys Val Ala Glu Ser Ala Gln Ser Pro Ala Ala  
 1605 1610 1615  
 Ala His Thr Thr Asp Thr Ala Gly Tyr Asn Ala Met Glu Glu Ser Val  
 1620 1625 1630  
 Ser Arg Glu Lys Pro Glu Leu Thr Ala Ser Thr Glu Arg Val Asn Lys  
 1635 1640 1645  
 Arg Met Ser Met Val Val Ser Gly Leu Thr Pro Glu Glu Phe Met Leu  
 1650 1655 1660  
 Val Tyr Lys Phe Ala Arg Lys His His Ile Thr Leu Thr Asn Leu Ile  
 1665 1670 1675 1680  
 Thr Glu Glu Thr Thr His Val Val Met Lys Thr Asp Ala Glu Phe Val  
 1685 1690 1695  
 Cys Glu Arg Thr Leu Lys Tyr Phe Leu Gly Ile Ala Gly Gly Lys Trp  
 1700 1705 1710  
 Val Val Ser Tyr Phe Trp Val Thr Gln Ser Ile Lys Glu Arg Lys Met  
 1715 1720 1725

Leu Asn Glu His Asp Phe Glu Val Arg Gly Asp Val Val Asn Gly Arg  
1730 1735 1740  
Asn His Gln Gly Pro Lys Arg Ala Arg Glu Ser Gln Asp Arg Lys Ile  
1745 1750 1755 1760  
Phe Arg Gly Leu Glu Ile Cys Cys Tyr Gly Pro Phe Thr Asn Met Pro  
1765 1770 1775  
Thr Asp Gln Leu Glu Trp Met Val Gln Leu Cys Gly Ala Ser Val Val  
1780 1785 1790  
Lys Glu Leu Ser Ser Phe Thr Leu Gly Thr Gly Val His Pro Ile Val  
1795 1800 1805  
Val Val Gln Pro Asp Ala Trp Thr Glu Asp Asn Gly Phe His Ala Ile  
1810 1815 1820  
Gly Gln Met Cys Glu Ala Pro Val Val Thr Arg Glu Trp Val Leu Asp  
1825 1830 1835 1840  
Ser Val Ala Leu Tyr Gln Cys Gln Glu Leu Asp Thr Tyr Leu Ile Pro  
1845 1850 1855  
Gln Ile Pro His Ser His Tyr  
1860

CLAIMS

What I claim is:

1. A method for detecting differential expression of at least one marker gene in pre-invasive cancerous breast tissue, said method comprising the steps of:
  - (a) obtaining an abnormal breast tissue sample by a collection step wherein said abnormal breast tissue sample comprises substantially exclusively abnormal breast tissue which exhibits histological or cytological characteristics of pre-invasive breast cancer;
  - (b) isolating mRNA from said abnormal breast tissue sample;
  - (c) preparing at least one abnormal breast tissue cDNA library from said mRNA isolated from said abnormal breast tissue sample;
  - (d) obtaining a normal breast tissue sample from humans either with or without disease, said normal breast tissue sample comprising substantially exclusively normal breast tissue which does not exhibit histological or cytological characteristics of pre-invasive breast cancer;
  - (e) preparing at least one normal breast tissue cDNA library from said normal breast tissue sample; and
  - (f) comparing said abnormal breast tissue cDNA library with said normal tissue cDNA library to determine whether the expression of at least one marker gene in said abnormal breast tissue sample is different from the expression of said marker gene in said normal breast tissue sample.
2. The method according to Claim 1 wherein said collection step is microscopically-directed.
3. The method according to Claim 2 wherein the size of said abnormal tissue sample substantially conforms to an isolatable tissue structure such that only cells exhibiting abnormal cytological or histological characteristics are collected.
4. The method according to Claim 3 wherein said isolatable tissue structure comprises ductal epithelial cells in pre-invasive breast cancer tissue.
5. The method according to Claim 1 further comprising confirming said differential expression of said marker gene in said normal tissue sample and in said abnormal tissue sample by using a hybridization or PCR technique.

6. The method according to Claim 5 wherein said hybridization technique comprises RT-PCR.

7. The method according to Claim 5 wherein said hybridization technique comprises nuclease protection assays.

8. The method according to Claim 5 wherein said hybridization technique comprises in-situ hybridization of RNA in said abnormal tissue sample and in said normal tissue sample.

9. The method according to Claim 1 wherein said abnormal cDNA library and said normal cDNA library are compared by means of differential display.

10. The method according to Claim 1 wherein said abnormal cDNA library and said normal cDNA library are compared by means of differential screening.

11. The method according to claim 1, wherein said normal tissue comprises normal breast tissue cells.

12. The method according to claim 1, wherein said abnormal breast tissue cells are non-comedo ductal carcinoma in situ cells.

13. The method according to claim 1, wherein the primer used in the PCR amplification technique is selected from the group consisting of randomly selected primers having the sequences

5'-CGCGACGGCGCGCGTCTGCCAGGG-3', 5'-CTTGGCGCGATACGCACAAAC-3',  
5'-AACCCCTACCCCTAACCCCAA-3', 5'-CGCCCCCTGGCTTACCCCTCCCCGGCG-3',  
5'-GGATGGCGTCCGTAAACCCGACGCT-3', 5'-ACTGGGCTGTCTGGGGTGGGGGGG-3',  
5'-CTGAGAGGTAGCCGCGGGAGGCTG-3', 5'-GCCTGGCCGGACACGGATTACCC-3',  
5'-TTAGGGCATGGTGGACCTGGAGACG-3', 5'-TGTGGTTACGTCAAGCGAAGGTAAATA-3',

15. A method of diagnosing the presence of pre-invasive breast cancer in human pathologic tissues, said method comprising the steps of:

- (a) obtaining an abnormal breast tissue sample by a collection step wherein said abnormal breast tissue sample comprises substantially exclusively abnormal breast tissue which exhibits histological or cytological characteristics of pre-invasive breast cancer;
- (b) isolating mRNA from said abnormal breast tissue sample;
- (c) preparing at least one abnormal breast tissue cDNA library from said mRNA isolated from said abnormal breast tissue sample;
- (d) obtaining a normal breast tissue sample from humans either with or without disease, said normal breast tissue sample comprising substantially exclusively normal breast tissue which does not exhibit histological or cytological characteristics of pre-invasive breast cancer;
- (e) preparing at least one normal breast tissue cDNA library from said normal breast tissue sample; and
- (f) comparing said abnormal breast tissue cDNA library with said normal tissue cDNA library to determine whether the expression of at least one marker gene in said abnormal breast tissue sample is different from the expression of said marker gene in said normal breast tissue sample.
- (g) cloning said differentially expressed marker gene using sequence-based amplification to create a cloned marker gene;
- (h) sequencing said cloned marker gene;
- (i) producing proteins encoded by said cloned marker gene;

18. The method according to claim 15, wherein said medical diagnostic tests comprise blood tests.

19. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding secreted proteins.

20. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding transcription factors.

21. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding RibRed.

22. The method according to claim 15, wherein said cloned marker genes encoding secreted proteins are employed in the diagnosis of specific diseases by using a blood test.

23. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences adapted to clone marker genes which encode cell surface proteins.

24. The method according to claim 15, wherein said proteins encoded by said cloned marker comprise cell surface proteins and wherein the presence of said proteins as a diagnostic indicator is detected by using a diagnostic imaging test.

25. A diagnostic method to determine the presence of pre-invasive breast cancer using detection of a differentially expressed marker gene, according to claim 15, wherein said diagnostic method comprises:

a) obtaining a substantially purified marker gene which is expressed to a greater degree in cells collected by a microscopically-directed cloning method from abnormal tissue than in cells collected from normal tissue;

b) probing tissues using a hybridization technique to determine whether said substantially purified marker gene is differentially expressed; and,

c) probing nucleic acids of tissues using a standard hybridization technique to determine the presence of said substantially purified marker gene in a tissue, the

presence of the marker gene indicating the presence of non-comedo DCIS which is pre-invasive breast cancer.

26. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:1, which comprises

```
TTGGGAATTG GGTACGGGGG CCCCCCACTG TGCCGAATTG CTGCATGGGG GGGATCCACT 60
AGTTCAGAGC AGGCCGCCAC CCGTAGGACT CCAGCTTTG TTCTGTTCCCT TTAGTGAGGG 120
TTAATTTCTG AGCTTGGCGT AATCATGGTC ATAGCTGTT CCTGTGTGAA ATTGTATCC 180
GCTCACAATT CCACACAACA TACGAGCCGG AAGCATAAAA GTGTAAAGCC TGGGGTGCCT 240
AATGAGTGAG CTAACTCACA TTAA 264
```

27. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:2, which comprises

```
TAGCCCGGTT ATCGAAATAG CCACAGCGCC TCTTCACTAT CAGCAGTACG CCGCCCACTT 60
GTACGGACAC GGA 73
```

28. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:3, which comprises

```
TGCCCGATGT GTGTCTACA ACTGGGGCTG TGGCTGATTG CGATAA 46
```

29. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:4, which comprises

```
TAGCCCATGA TTCTGTGCC GTACAACTGG GGCCTGTGG CTGATTGCA TANNNNNAGC 60
ATCAGCCCCGA CG 72
```

30. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:5, which comprises

```
TAGCCCGGTT ATCGAAATCA GCCACAGGGC CTAACCTCTG CAGAACCTT TGACCATCAC 60
CAGTTGTACG GACACGAAC TATC 84
```

31. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:6, which comprises

```
GTGGTTCCG AAATTCCTGG GAAGGGGGGT GCTGGCGTGT GGAATTGTGG CGGCCCTGG 60
TCTGCCGCCGG CGTTTTTGT CTACATTCTG CGTAGCTCG 99
```

32. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:7, which comprises

ATCAGCGCGC GACATTCGGG TACCCCGCGCC CCCCCCTCCG TCGGAATTCC TCGAGCCGGG 60  
ATCCATAGGA TGTGGAGTTA GTTTTGTT 88

33. A method for detecting differential expression of at least one marker gene in pre-invasive cancerous breast tissue, said method comprising the steps of:

- (a) obtaining an abnormal tissue sample by a collection step wherein said abnormal tissue sample comprises substantially exclusively abnormal tissue which exhibits histological or cytological characteristics of pre-invasive cancer;
- (b) isolating mRNA from said abnormal tissue sample;
- (c) preparing at least one abnormal tissue cDNA library from said mRNA isolated from said abnormal tissue sample;
- (d) obtaining a normal tissue sample from humans either with or without disease, said normal tissue sample comprising substantially exclusively normal tissue which does not exhibit histological or cytological characteristics of pre-invasive cancer;
- (e) preparing at least one normal tissue cDNA library from said normal tissue sample; and
- (f) comparing said abnormal tissue cDNA library with said normal tissue cDNA library to determine whether the expression of at least one marker gene in said abnormal tissue sample is different from the expression of said marker gene in said normal tissue sample.

34. The method according to Claim 33 wherein said collection step is microscopically-directed.

- a) obtaining a substantially purified marker gene which is expressed to a greater degree in cells collected by a microscopically-directed cloning method from abnormal tissue than in cells collected from normal tissue;
- b) probing tissues using a hybridization technique to determine whether the marker gene is differentially expressed; and,
- c) probing nucleic acids of tissues using a standard PCR technique to determine the presence of the marker gene in a tissue, the presence of the marker gene indicating the presence of pre-invasive cancer.

35. Substantially purified DNA having the nucleotide sequences selected from the group of sequences consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

36. An expression vector for the differentially expressed polypeptides encoded by said substantially purified DNA comprising one of the group of DNA sequences of claim 28 operatively linked to at least one control sequence compatible with a suitable bacterial host cell.

37. The vector of claim 36 wherein the DNA encoding the differentially expressed polypeptides encoded by said substantially purified DNA comprising one of the group of DNA sequences of claim 28 is linked to at least one sequence from bacteriophage.

38. Substantially purified polypeptides encoded by substantially purified DNA comprising one of the group of DNA sequences of claim 35 free of proteins other than proteins encoded by said substantially purified DNA.

39. An antibody specifically binding one of the group of polypeptides encoded by one of the nucleotide sequences selected from the group of sequences consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

40. A method of detecting and/or determining said antibody in a test sample, comprising the steps:

- (a) providing a test sample suspected of containing said marker protein;
- (b) adding a quantity of said marker protein of claim 38 to the antibody of claim 39; and
- (c) determining a level of said marker protein in said test sample.

41. A method of screening compounds for activity in the treatment of breast cancer, comprising the steps of:

- (a) ligating a DNA sequence that regulates expression of the BRCA1 gene into a vector, the vector having a reporter gene, so that the DNA sequence is located such that the DNA sequence regulates expression of the reporter gene;

(b) introducing the ligated DNA sequence/reporter gene into a breast cancer cell;

(c) administering a compound to the breast cancer cell; and

(d) detecting levels of a protein produced by the reporter cell.

42. The method according to claim 41 wherein the DNA sequence is as essentially set forth in SEQ ID NO:48.

43. The method according to claim 42 wherein the DNA sequence is selected from among:

a. a DNA sequence which hybridizes to SEQ ID NO:48 or fragments thereof; and

b. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

44. The method according to claim 41 wherein the ligated DNA sequence/reporter gene is introduced into the breast cancer cell by cloning the ligated DNA sequence/reporter gene into an expression vector and transfecting the breast cancer cells with the expression vector.

45. The method according to claim 44 wherein the DNA sequence is essentially set forth in SEQ ID NO:48 or its complementary strands.

46. A method of producing an indicator compound, comprising the steps of:

(a) ligating a DNA sequence that regulates expression of the BRCA1 gene into a vector, the vector having a reporter gene, so that the DNA sequence is located such that the DNA sequence regulates expression of the reporter gene;

(b) introducing the ligated DNA sequence/reporter gene into a breast cancer cell;

(c) administering a biological agent to the breast cancer cell; and

(d) producing a protein encoded by the reporter gene; and

(e) reacting the protein encoded by the reporter gene with a compound in the reaction media to produce the indicator compound.

47. The method according to claim 46 wherein the ligated DNA sequence/reporter gene is introduced into the breast cancer cell by cloning the ligated

DNA sequence/reporter gene into an expression vector and transfecting the breast cancer cells with the expression vector.

48. The method according to claim 46 wherein the DNA sequence is as essentially set forth in SEQ ID NO:48 or its complementary strands.

49. The method according to claim 46 wherein the DNA sequence is selected from among:

- a. a DNA sequence which hybridizes to SEQ ID NO:48 or fragments thereof; and
- b. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

50. A method of treating breast cancer in a patient comprising the steps of ligating a gene that encodes a protein having an amino acid sequence as essentially set forth in SEQ ID NO:49 with a promoter capable of inducing expression of the gene in a breast cancer cell and introducing the ligated gene into a breast cancer cell.

51. The method of treating breast cancer described in claim 50 wherein the gene has a DNA sequence selected from among:

- a. the DNA sequence as essentially set forth in SEQ ID NO:47 or its complementary strands;
- b. a DNA sequence which hybridizes to SEQ ID NO:47 or fragments thereof; and
- c. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

52. The method of treating breast cancer described in claim 50 wherein the gene has a DNA sequence having 20-99% homology with SEQ ID NO:47.

53. The method according to claim 50 wherein the ligated gene is introduced into the cell in a viral expression vector.

54. The method according to claim 50 wherein the breast cancer is gene-linked hereditary breast cancer.

55. The method described in claim 50 wherein the breast cancer is sporadic breast cancer.

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AMENDED CLAIMS

[received by the International Bureau on 14 June 1995 (14.06.95); original claims 13 and 15 amended; new claims 14,16 and 17 added; remaining claims unchanged (8 pages)]

6. The method according to Claim 5 wherein said hybridization technique comprises RT-PCR.

5 7. The method according to Claim 5 wherein said hybridization technique comprises nuclease protection assays.

8. The method according to Claim 5 wherein said hybridization technique comprises in-situ hybridization of RNA in said abnormal tissue sample and in said normal tissue sample.

10 9. The method according to Claim 1 wherein said abnormal cDNA library and said normal cDNA library are compared by means of differential display.

10. The method according to Claim 1 wherein said abnormal cDNA library and said normal cDNA library are compared by means of differential screening.

11. The method according to claim 1, wherein said normal tissue comprises normal breast tissue cells.

15 12. The method according to claim 1, wherein said abnormal breast tissue cells are non-comedo ductal carcinoma in situ cells.

13. The method according to claim 1, wherein the primer used in the PCR amplification technique is selected from the group consisting of randomly selected primers having the sequences

20 5'-CGCGACGGCCGGCGCTGCCAGGG-3', 5'-CTTGGCGCATAACGACAAAC-3',  
5'-AACCTCACCTAACCCCAA-3', 5'-CGCCCCCTGCCTTACCCCTCCCCGCCG-3',

5'-GGATGGCGCTCTGTAAACCGACGCT-3', 5'-ACTGGGCTGTCTGCCGTGGGGGG-3',  
5'-CTGAGAGGTAGCCGCGCGAGGCTG-3', 5'-GCCTGGCCGGACACGGATTACCGC-3',

5'-TTAGCGCATGGTGGACCTGGAGACG-3', 5'-TGTGGTTACGTCAGCGAAGGTAATA-3',  
5'-AGTCGCACCCATGTACCGCTCCGCC-3', 5'-TATCCAAGGGCAGGCTACGAGGCC-3',

5'-GGCCCGCCCCACGGTCTGGTATCTA-3', 5'-CTCCCTCCCCGGACTGGGGTTAGT-3',  
5'-ATGGGGGGCTCGGGCTGGTGC-3', 5'-CGTGAAGCTATGCCCTCCCTCAAC-3',

5'-GTGCCGTCTAGCCCTCAGCGATC-3', 5'-GCCACACTAGGCTCCGGAGGGAGGG-3',  
5'-TGGGCCAGGCCCTCCGGGGCCGGTAT-3', 5'-CCGGAACTGCGATAGCGTCCGTCCC-3',

5'-AGCGGACACCTGTTTCCCAGAGCC-3', 5'-AACGGGTGGACATCCGCTGCCGCC-3',  
5'-TGAACCACCGATGTCATCGTCCGA-3', 5'-TCATCCCCGGAAAGACGCTGCC-3',

5'-ATAGGCTGCGGCACGCGCTGGGACT-3', 5'-GACCAAGGTGGCACGAGCATGACA-3',  
5'-AGCGTAGTCATCGGCCCTCGCGCCC-3', 5'-GGCCCTAGCCCAAGGGTGAAGCCCA-3',

5'-CCCAGTGTACGGGCCGCCCAAGC-3', 5'-CCTTCTGGTTACCTGCCCTCGGG-3',  
5'-TCCGGACAGCAGCCACGCCAAGGGC-3', 5'-ACGGCTGGTCCACCGAGGGCTGAT-3',

5'-CGATGCAAGGCCAGCAGCACTCGAC-3', 5'-CCCCGGAGCGGACCCGGACGTG-3',  
5'-AGCGGGGAGGGATCGGGGCCAAGC-3', 5'-GCCTGGTGTAGGCAGGCAGCTTTA-3',

5'-CCACCCCTGTAGTGCCTGGCTGCCAG-3', 5'-GGAACCCGAGCCCCGTCCAGGGTTC-3',

5'-TCGGGCAGCAAGGCCGGGACGCTCC-3', 5'-GACGGGGGACGGGCTAGGTGGCTTA-3',  
and 5'-CTTGTGCGCGGAGAGGGCTGCC-3'.

14. The method according to claim 2, wherein said abnormal tissue sample is approximately 2 mm in diameter.

5 15. A method of diagnosing the presence of pre-invasive breast cancer in human pathologic tissues, said method comprising the steps of:

10 (a) obtaining an abnormal breast tissue sample by a collection step wherein said abnormal breast tissue sample comprises substantially exclusively abnormal breast tissue which exhibits histological or cytological characteristics of pre-invasive breast cancer;

(b) isolating mRNA from said abnormal breast tissue sample;

15 (c) preparing at least one abnormal breast tissue cDNA library from said mRNA isolated from said abnormal breast tissue sample;

(d) obtaining a normal breast tissue sample from humans either with or without disease, said normal breast tissue sample comprising substantially exclusively normal breast tissue which does not exhibit histological or cytological characteristics of pre-invasive breast cancer;

20 (e) preparing at least one normal breast tissue cDNA library from said normal breast tissue sample; and

25 (f) comparing said abnormal breast tissue cDNA library with said normal tissue cDNA library to determine whether the expression of at least one marker gene in said abnormal breast tissue sample is different from the expression of said marker gene in said normal breast tissue sample.

(g) cloning said differentially expressed marker gene using sequence-based amplification to create a cloned marker gene;

(h) sequencing said cloned marker gene;

(i) producing proteins encoded by said cloned marker gene;

(j) generating antibodies which will recognize said proteins encoded by said cloned marker gene by antigen recognition; and

30 (k) detecting said recognized antigen by means of medical diagnostic tests.

16. The method according to claim 15, wherein said medical diagnostic tests comprise diagnostic tissue tests.

17. The method according to claim 15, wherein said medical diagnostic tests comprise X-ray tests.

18. The method according to claim 15, wherein said medical diagnostic tests comprise blood tests..

5 19. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding secreted proteins.

10 20. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding transcription factors.

21. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences designed to clone marker genes encoding RibRed.

15 22. The method according to claim 15, wherein said cloned marker genes encoding secreted proteins are employed in the diagnosis of specific diseases by using a blood test.

23. The method according to claim 15, wherein said sequence-based amplification employs DNA sequences adapted to clone marker genes which encode cell surface proteins.

20 24. The method according to claim 15, wherein said proteins encoded by said cloned marker comprise cell surface proteins and wherein the presence of said proteins as a diagnostic indicator is detected by using a diagnostic imaging test.

25 25. A diagnostic method to determine the presence of pre-invasive breast cancer using detection of a differentially expressed marker gene, according to claim 15, wherein said diagnostic method comprises:

a) obtaining a substantially purified marker gene which is expressed to a greater degree in cells collected by a microscopically-directed cloning method from abnormal tissue than in cells collected from normal tissue;

30 b) probing tissues using a hybridization technique to determine whether said substantially purified marker gene is differentially expressed; and,

c) probing nucleic acids of tissues using a standard hybridization technique

to determine the presence of said substantially purified marker gene in a tissue, the presence of the marker gene indicating the presence of non-comedo DCIS which is pre-invasive breast cancer.

5 26. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:1, which comprises

10 TTGGGAATTG GGTACGGCGGG CCCCCCACTG TGCCGAATTG CTGCATGGG GGGATCCACT 60  
AGTCAGAGC AGGCCGCCAC CCGTAGGACT CCAGCTTTG TTCGTTCCCT TTAGTGAGGG 120  
TTAATTTCG AGCTTGGCGT AATCATGGTC ATAGCTGTGAA CCTGTGTGAA ATTGTATCC 180  
GCTCACAAATT CCACACAAACA TACGAGCCGG AAGCATAAAA GTGAAAGCC TGGGGTGCCT 240  
AATGAGTGAG CTAACTCACA TTAA 264

27. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:2, which comprises

15 TAGCCCCGTT ATCGAAATAG CCACAGCGCC TCTTCACTAT CAGCAGTACG CGCCCCAGTT 60  
GTACGGACAC GGA 73

28. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:3, which comprises

20 TGCCCCATGT GTGTCGTACA ACTGGCGCTG TGGCTGATTT CGATAA 46

29. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:4, which comprises

25 TAGCCCCATGA GTTCGTGTCC GTACAACTGG GGCCTGTGG CTGATTTCGA TANNNNNAGC 60  
ATCAGCCCCGA CG 72

30 30. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:5, which comprises

30 TAGCCCCGGTT ATCGAAATCA GGCACAGCGC CTAACCTCTG CAGAAGCCTT TGACCATCAC 60  
CAGTTGTACG GACACGAACG CATC 84

35 31. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:6, which comprises

GTCGGTTTCCG AAATTCCTGG GAAGGGGGGT GCTGGCGTGT GGAATTGTGG CGGGCCCTGG 60  
TCTGCCGCGG CGTTTTTGT CTACATTCTGT CGTAGCTCG 99

32. The method according to claim 25, wherein said substantially purified marker gene has the sequence listed according to SEQ ID NO:7, which comprises

5 ATCAGGGCGC GACATTEGGG TACCGCGGCC CCCCCCTCCG TCGGAATTCC TCGAGCCGGG 60  
ATCCATAGGA TGTGGAGTTA GTTTTGT 88

33. A method for detecting differential expression of at least one marker gene in pre-invasive cancerous breast tissue, said method comprising the steps of:

10 (a) obtaining an abnormal tissue sample by a collection step wherein said abnormal tissue sample comprises substantially exclusively abnormal tissue which exhibits histological or cytological characteristics of pre-invasive cancer;

(b) isolating mRNA from said abnormal tissue sample;

(c) preparing at least one abnormal tissue cDNA library from said mRNA isolated from said abnormal tissue sample;

15 (d) obtaining a normal tissue sample from humans either with or without disease, said normal tissue sample comprising substantially exclusively normal tissue which does not exhibit histological or cytological characteristics of pre-invasive cancer;

(e) preparing at least one normal tissue cDNA library from said normal tissue sample; and

20 (f) comparing said abnormal tissue cDNA library with said normal tissue cDNA library to determine whether the expression of at least one marker gene in said abnormal tissue sample is different from the expression of said marker gene in said normal tissue sample.

25 34. The method according to Claim 33 wherein said collection step is microscopically-directed.

a) obtaining a substantially purified marker gene which is expressed to a greater degree in cells collected by a microscopically-directed cloning method from abnormal tissue than in cells collected from normal tissue;

b) probing tissues using a hybridization technique to determine whether the marker gene is differentially expressed; and,

30 c) probing nucleic acids of tissues using a standard PCR technique to determine the presence of the marker gene in a tissue, the presence of the marker gene indicating the presence of pre-invasive cancer.

35. Substantially purified DNA having the nucleotide sequences selected from the group of sequences consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

5 36. An expression vector for the differentially expressed polypeptides encoded by said substantially purified DNA comprising one of the group of DNA sequences of claim 28 operatively linked to at least one control sequence compatible with a suitable bacterial host cell.

10 37. The vector of claim 36 wherein the DNA encoding the differentially expressed polypeptides encoded by said substantially purified DNA comprising one of the group of DNA sequences of claim 28 is linked to at least one sequence from bacteriophage.

15 38. Substantially purified polypeptides encoded by substantially purified DNA comprising one of the group of DNA sequences of claim 35 free of proteins other than proteins encoded by said substantially purified DNA.

39. An antibody specifically binding one of the group of polypeptides encoded by one of the nucleotide sequences selected from the group of sequences consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

20 40. A method of detecting and/or determining said antibody in a test sample, comprising the steps:

(a) providing a test sample suspected of containing said marker protein;  
(b) adding a quantity of said marker protein of claim 38 to the antibody of claim 39; and  
(c) determining a level of said marker protein in said test sample.

25 41. A method of screening compounds for activity in the treatment of breast cancer, comprising the steps of:

(a) ligating a DNA sequence that regulates expression of the BRCA1 gene into a vector, the vector having a reporter gene, so that the DNA sequence is located such that the DNA sequence regulates expression of the reporter gene;  
(b) introducing the ligated DNA sequence/reporter gene into a breast cancer

cell;

- (c) administering a compound to the breast cancer cell; and
- (d) detecting levels of a protein produced by the reporter cell.

42. The method according to claim 41 wherein the DNA sequence is as  
5 essentially set forth in SEQ ID NO:48.

43. The method according to claim 42 wherein the DNA sequence  
is selected from among:

- a. a DNA sequence which hybridizes to SEQ ID NO:48 or fragments thereof; and
- b. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

44. The method according to claim 41 wherein the ligated DNA sequence/reporter gene is introduced into the breast cancer cell by cloning the ligated DNA sequence/reporter gene into an expression vector and transfecting the breast  
10 cancer cells with the expression vector.

45. The method according to claim 44 wherein the DNA sequence is  
essentially set forth in SEQ ID NO:48 or its complementary strands.

46. A method of producing an indicator compound, comprising the steps of:

- (a) ligating a DNA sequence that regulates expression of the BRCA1 gene into a vector, the vector having a reporter gene, so that the DNA sequence is located such that the DNA sequence regulates expression of the reporter gene;
- (b) introducing the ligated DNA sequence/reporter gene into a breast cancer cell;
- (c) administering a biological agent to the breast cancer cell; and
- (d) producing a protein encoded by the reporter gene; and
- (e) reacting the protein encoded by the reporter gene with a compound in the reaction media to produce the indicator compound.

47. The method according to claim 46 wherein the ligated DNA sequence/reporter gene is introduced into the breast cancer cell by cloning the ligated DNA sequence/reporter gene into an expression vector and transfecting the breast  
20 cancer cells with the expression vector.

cancer cells with the expression vector.

48. The method according to claim 46 wherein the DNA sequence is as essentially set forth in SEQ ID NO:48 or its complementary strands.

49. The method according to claim 46 wherein the DNA sequence is selected from among:

- a. a DNA sequence which hybridizes to SEQ ID NO:48 or fragments thereof; and
- b. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

50. A method of treating breast cancer in a patient comprising the steps of ligating a gene that encodes a protein having an amino acid sequence as essentially set forth in SEQ ID NO:49 with a promoter capable of inducing expression of the gene in a breast cancer cell and introducing the ligated gene into a breast cancer cell.

51. The method of treating breast cancer described in claim 50 wherein the gene has a DNA sequence selected from among:

- a. the DNA sequence as essentially set forth in SEQ ID NO:47 or its complementary strands;
- b. a DNA sequence which hybridizes to SEQ ID NO:47 or fragments thereof; and
- c. DNA sequences which but for the degeneracy of the genetic code would hybridize to the DNA sequences defined in (a) and (b).

52. The method of treating breast cancer described in claim 50 wherein the gene has a DNA sequence having 20-99% homology with SEQ ID NO:47.

53. The method according to claim 50 wherein the ligated gene is introduced into the cell in a viral expression vector.

54. The method according to claim 50 wherein the breast cancer is gene-linked hereditary breast cancer.

55. The method described in claim 50 wherein the breast cancer is sporadic breast cancer.

## STATEMENT UNDER ARTICLE 19

Pursuant to Article 19 of the Patent Cooperation Treaty and Rule 46, Applicant respectfully submits the attached sheets of amended claims. The sheets are replacement sheets for pages 98-105 of the above referenced International application. These sheets contain Claims 6-55 of the above referenced international application. New Claims 14, 16 and 17 have been added to replacement pages 99-100. Additional primers have been listed in Claim 13 on replacement pages 98-99. These primers are described in the Sequence Listing. Claim 15 has been amended to include steps (j) and (k) on replacement page 99. The new claims and the amended claims do not go beyond the scope of the application as filed. The remaining replacement sheets include no amendments, but are filed to maintain the correct numbering of the claim pages.

Figure 1:

TABLE I: Anatomic Lesion Types in the Human Breast with Pre-malignant Implication

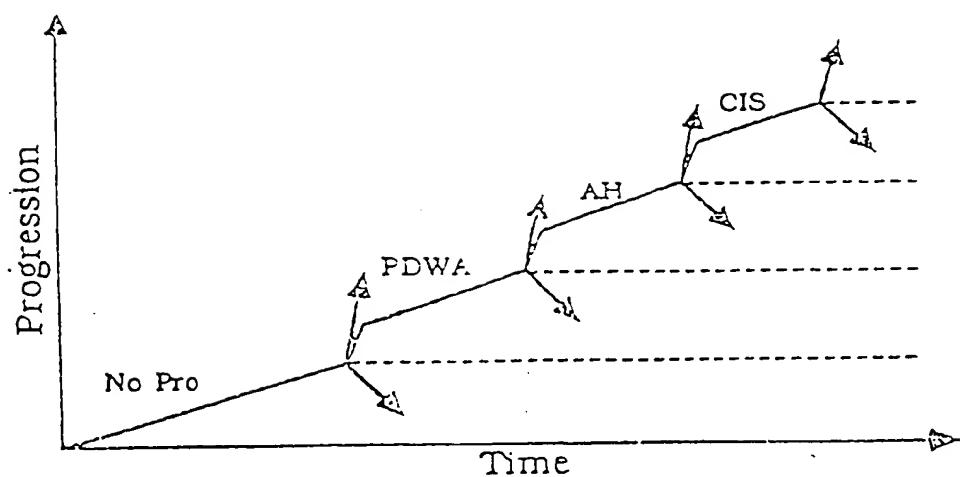
<u>Pre-malignant Lesions</u>	<u>Relative Risk*</u>	<u>P value</u>	<u>Reference</u>
Indicators of generalized increased risk			
Atypical ductal hyperplasia	4.5 fold	< .00001	(Dupont, et al, 1985 and 1993.)
Lobular CIS	9-10 fold	< .00001	(Page, et al, 1991.)
Determinant Lesions with Regional Risk			
Non-comedo DCIS	10-11 fold	< .00005	(Page, et al, 1982.)

\* represents the 95% confidence interval for relative risk.

Figure 1: Table I describes anatomic lesion types in the human breast with pre-malignant implication.

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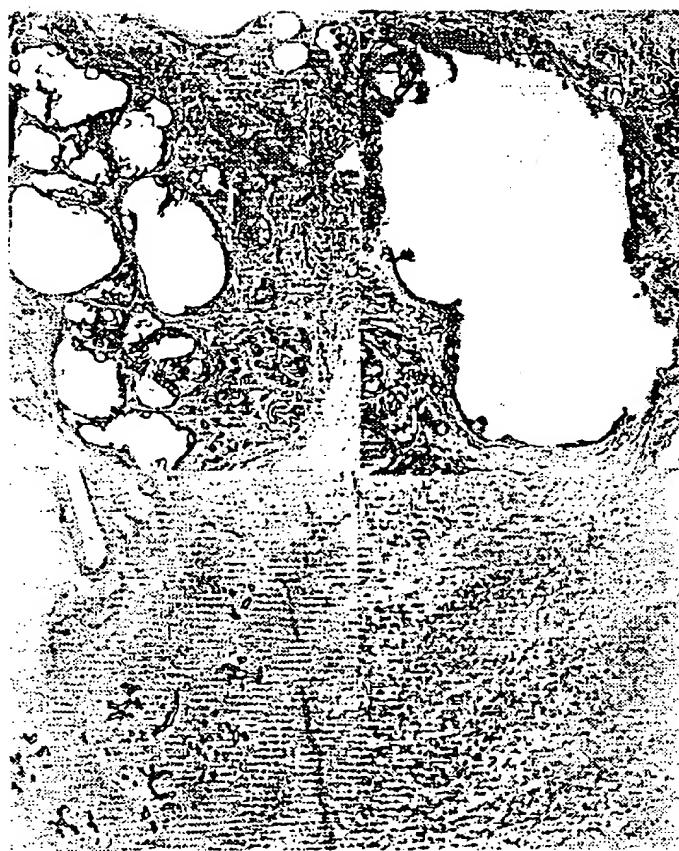
Fig. 2



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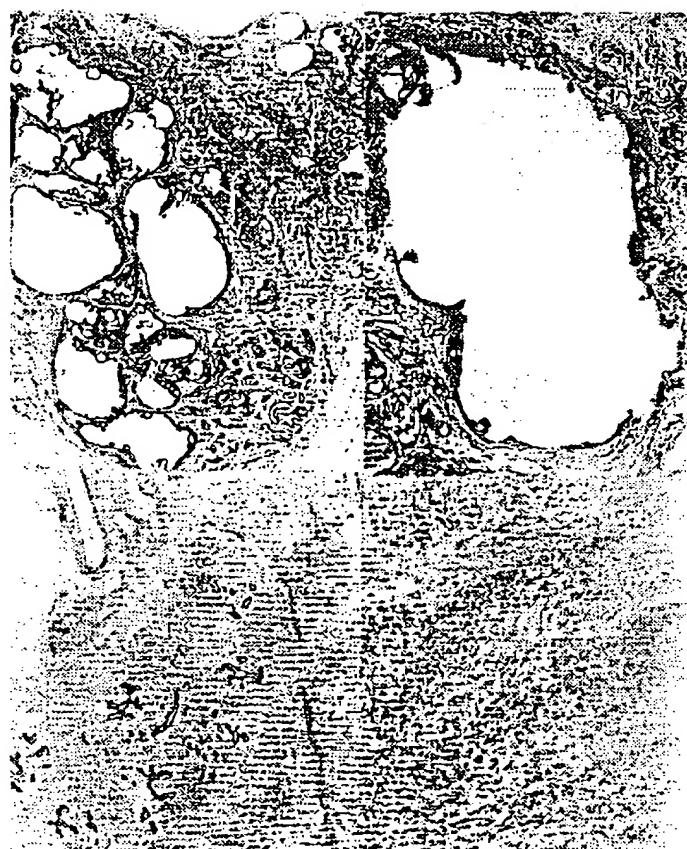
Fig. 3



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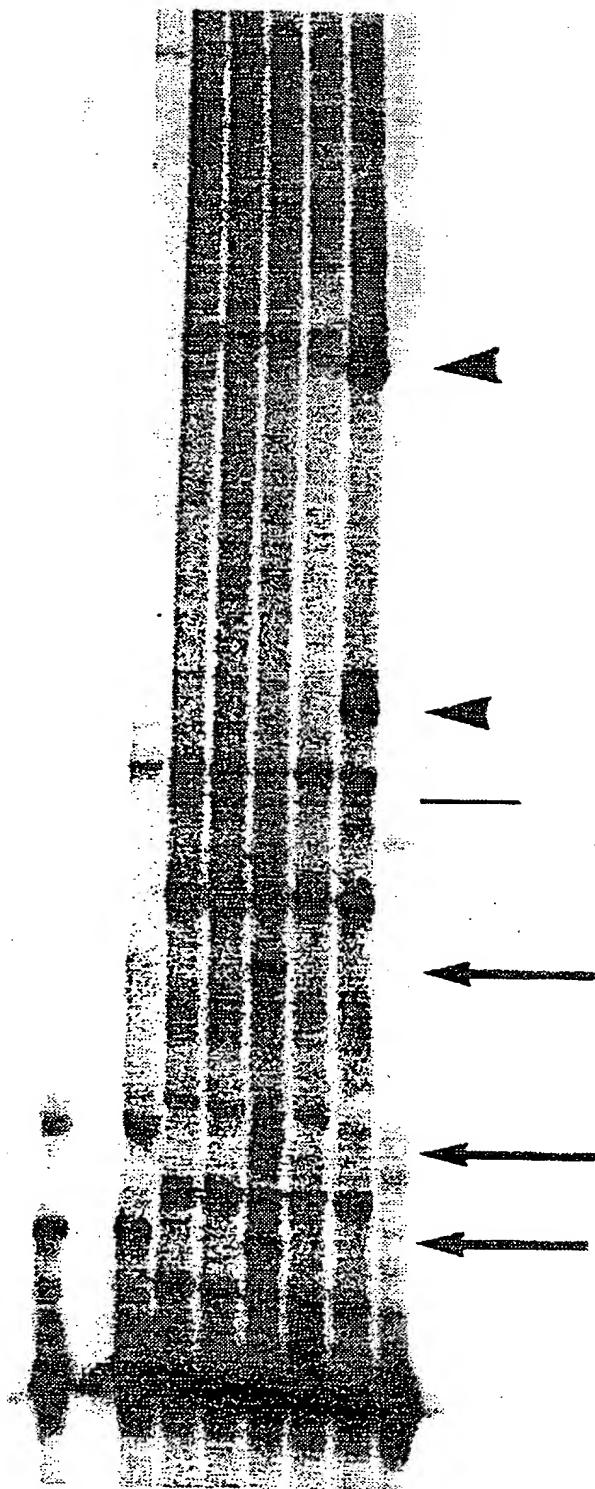
Fig. 3



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Fig. 5



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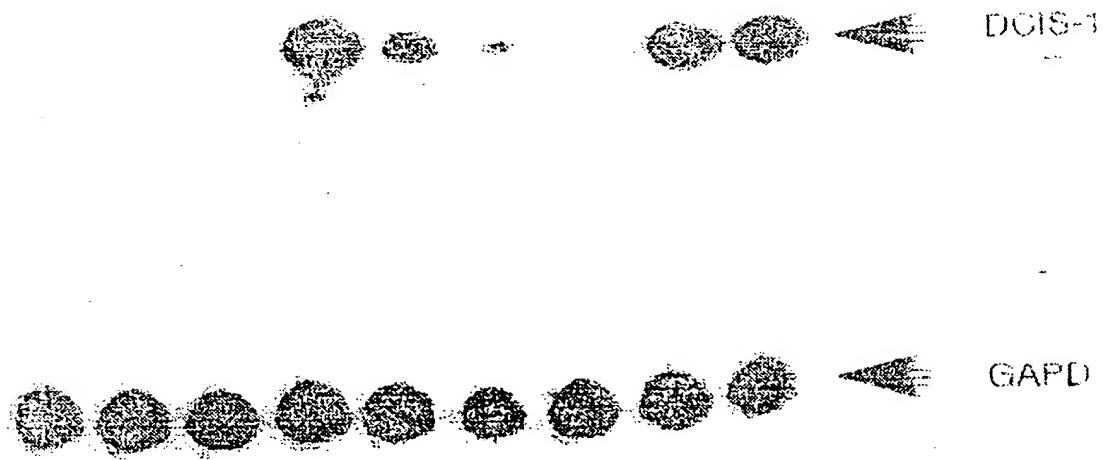
Human	TTCTCCTGACCACCAATGGGAGCCAATTACAATTAC
Hamster	
Hamster	TTCTGTTACCACTGATGGCAGCTAATGAA--AATGC--
Human	TAAGTGACTAAAGTAAGTTAAACTTGTGTAGACTAAGCAT
Hamster	
Hamster	--AAGTGACTCAG---AAGTTA-----GTGTT-----AGCAT
DCIS-1	GGGGGATCCACTAGTT--AGAGCAGGCCGCCACCCG
Hamster	
Hamster	GGGGGATCCACTAGTTCTAGAGCGG--CCGCCACCGC
DCIS-1	TAGGACTCCAGCTTTGTTCCCTCTAGTGAAGGGTTAA
Hamster	
Hamster	TGGAGCTCCAGCTTTGTTCCCTTAGTGA--GGGTTAA

Figure 6: Comparison of the sequence between DCIS-1 and the human and hamster genes.

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Fig. 7

Con NL1 NL2 NL3 #12 #6 #4 #8 #10 #10C



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Fig. 8 - Table of the Genetic Code

<u>Amino Acids</u>			<u>Codons</u>					
Alanine	Ala	A	GCA	GCC	GCG	GCU		
Cysteine	Cys	C	UGC	UGU				
Aspartic acid	Asp	D	GAC	GAU				
Glutamic acid	Glu	E	GAA	GAG				
Phenylalanine	Phe	F	UUC	UUU				
Glycine	Gly	G	GGA	GGC	GGG	GGU		
Histidine	His	H	CAC	CAU				
Isoleucine	Ile	I	AUA	AUC	AUU			
Lysine	Lys	K	AAF	AAG				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU
Methionine	Met	M	AUG					
Asparagine	Asn	N	AAC	AAU				
Proline	Pro	P	CCG	CCC	CCG	CCU		
Glutamine	Gln	Q	CAA	CAG				
Arginine	Arg	R	AGA	AGG	CGA	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU		
Valine	Val	V	GUA	GUC	GUG	GUU		
Tryptophan	Trp	W	UGG					
Tyrosine	Tyr	Y	UAC	UAU				

Figure 8: Table of the Genetic Code.

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## SEQ ID NO: 1: (DCIS-1)

TGGGAATTG GGTACGGGG CCCCCCACTG TGCGAATTG CTGCATGGG GGGATCCACT 60  
AGTTTCAAGAGC CCGTAGGACT CCAGCTTTG TTCTGTCCTT TTAGTGAGGG TAAATTTCG 120  
AGCTTGGCGT AATCATGGTC ATCCCTGTG 60 AAATTGTAT CCGCTCACAA TTCCACACAA 180  
CATACGAGCC GGAAGCATAA AAGTGTAAAGC AATGAGTGAG CTAACTCACA TTAA 234

## SEQ ID NO: 2: (DCIS-2)

TAGCCCCGTT ATCGAAATAG CCACACGGCC TCTTCACAT CACCGAGTACG CCCCCCACTT 60  
GTA CGG ACA CGG 72

## SEQ ID NO: 3: (DCIS-3)

TGCCCCATGA GTTGTGTCGT ACAACTGGCG CTGTGGCTGA TTTCGATAA 49

## SEQ ID NO: 4: (DCIS-4)

TAGCCCCATGA GTTGTGTCGT ACAACTGGCG CTGTGGCTGA TANNNNNAGC 60  
ATCAGCCCCGA CG 72

## SEQ ID NO: 5: (DCIS-5)

TAGCCCCGTT ATCGAAAATCA GCGAACAGGGC CTAACCTCTG CAGAAGCCTT TGACCATCAC 60  
CACTTGTAACG GAAACGAAC TATC 84

## SEQ ID NO: 6: (DCIS-6)

GTGGTTTCCG AAATTCTCG GGAAGGGGGG TGCTGGGCG TGGAATTGTC CGGGCCCCCTG 60  
GTCTGGCGG CGCTTTTTT GCTACATTC GTCGTACCTC G 101

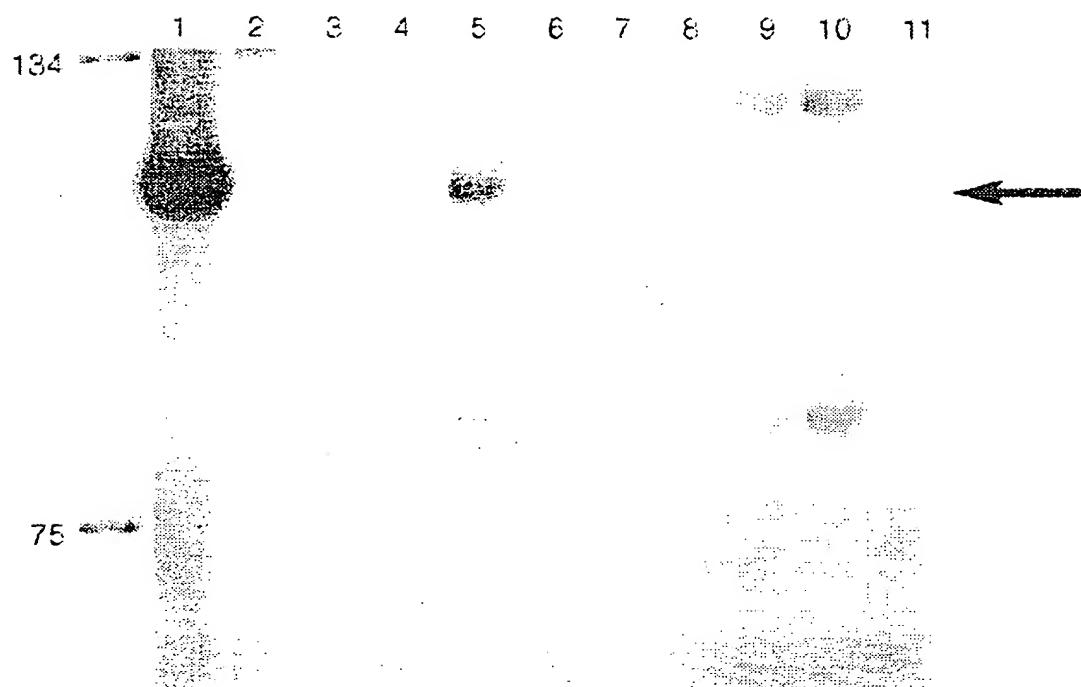
## SEQ ID NO: 7: (DCIS-7)

ATCAGCGCCG GACATTCGGG TACCCGGGCC C\*\*\*\*\*TCCG TCGGAATTCC TCGAGCCGGG 60  
AT\*\*ATAGGA TGTGGAGTTA GTTTTGT 88

Figure 9: Table of Differentially Expressed Marker Genes From Pre-Invasive Human Breast Tissue

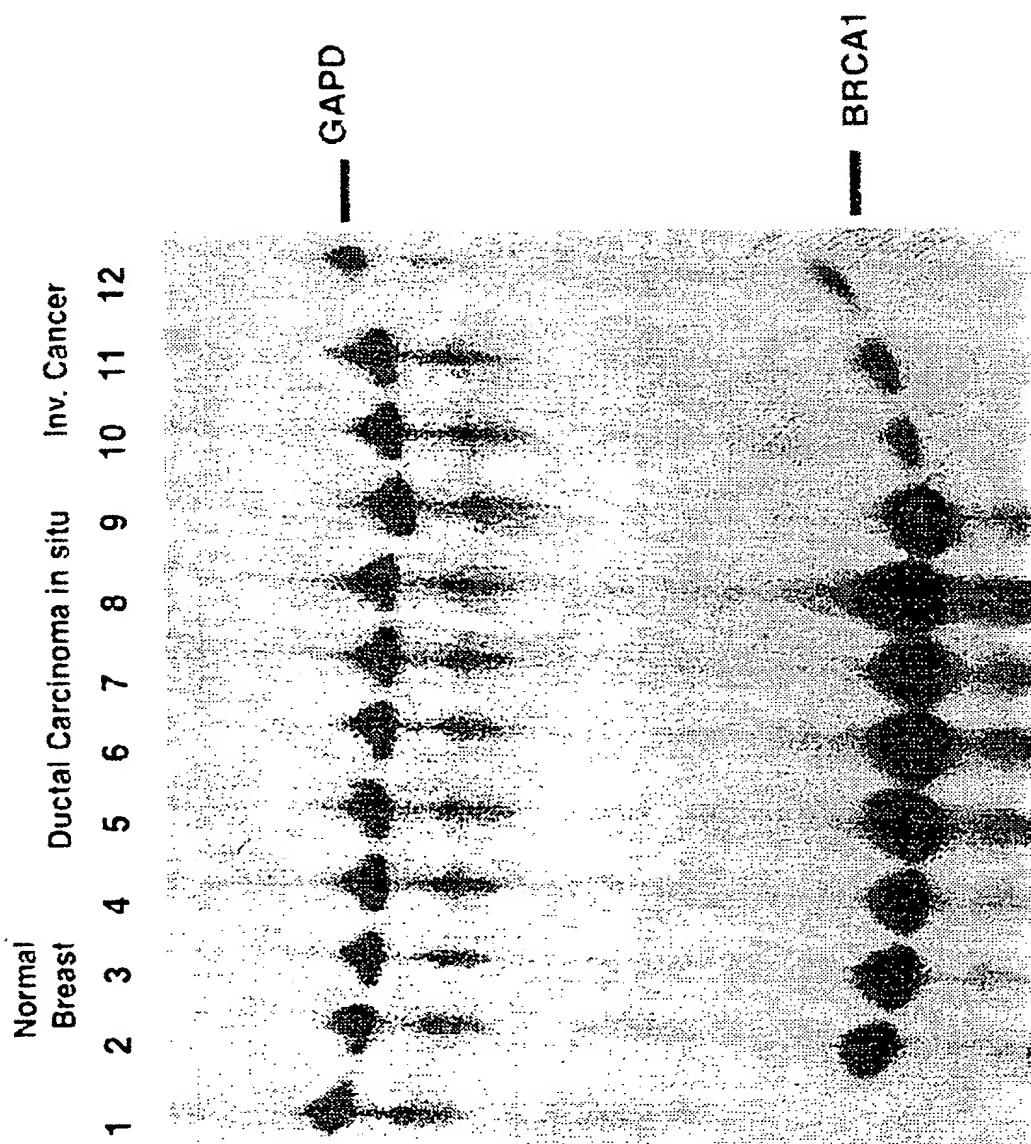
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Fig. 10A



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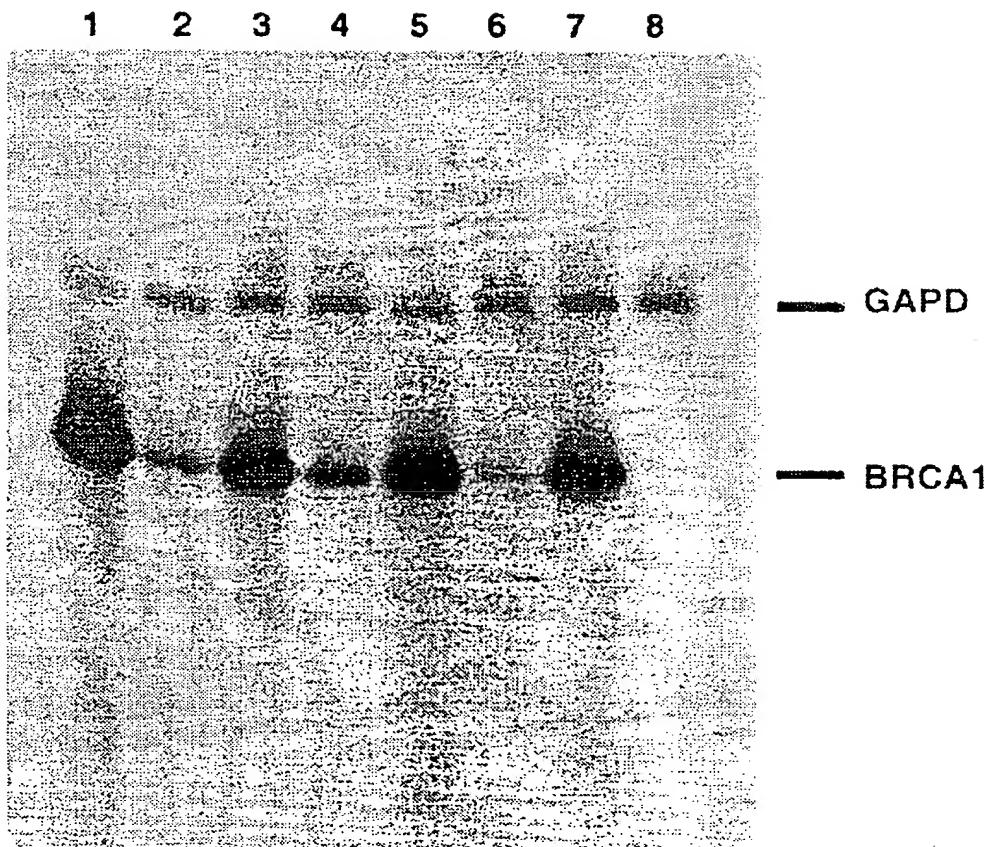
Fig. 10B



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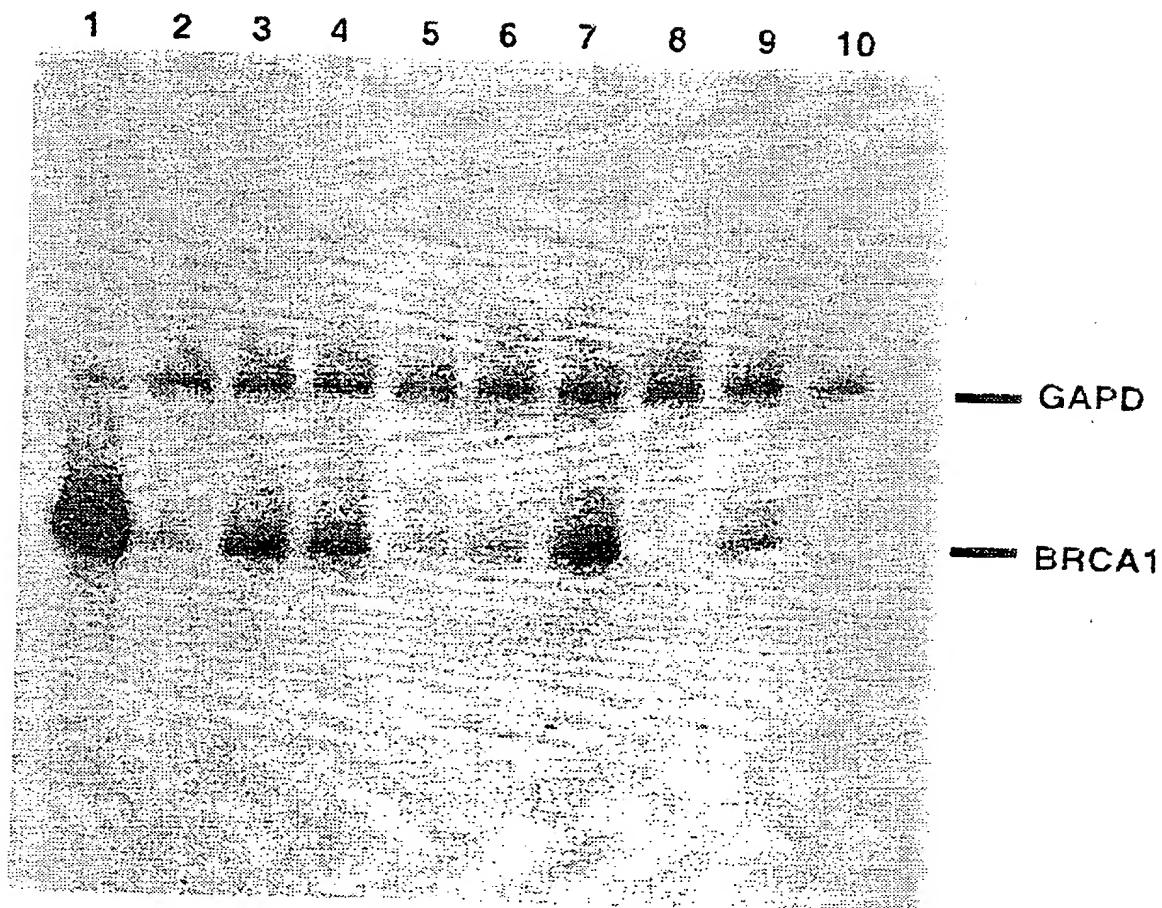
Fig. 11A



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Fig. 11B



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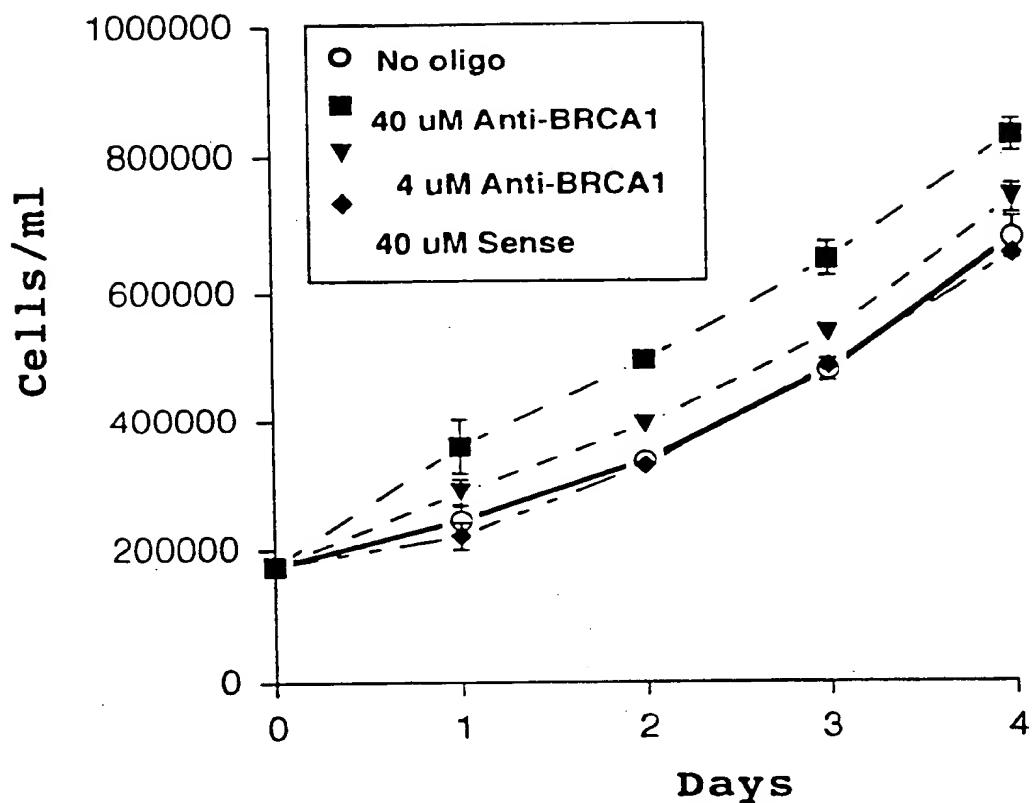


Fig. 12A

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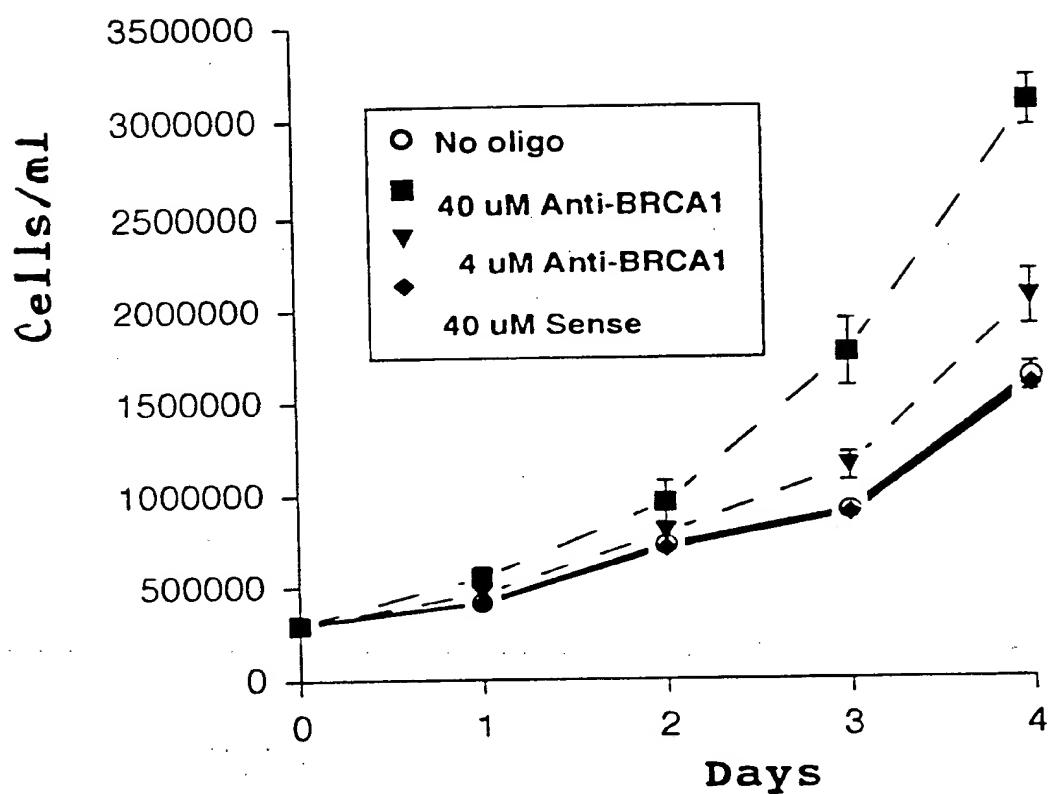


Fig. 12B

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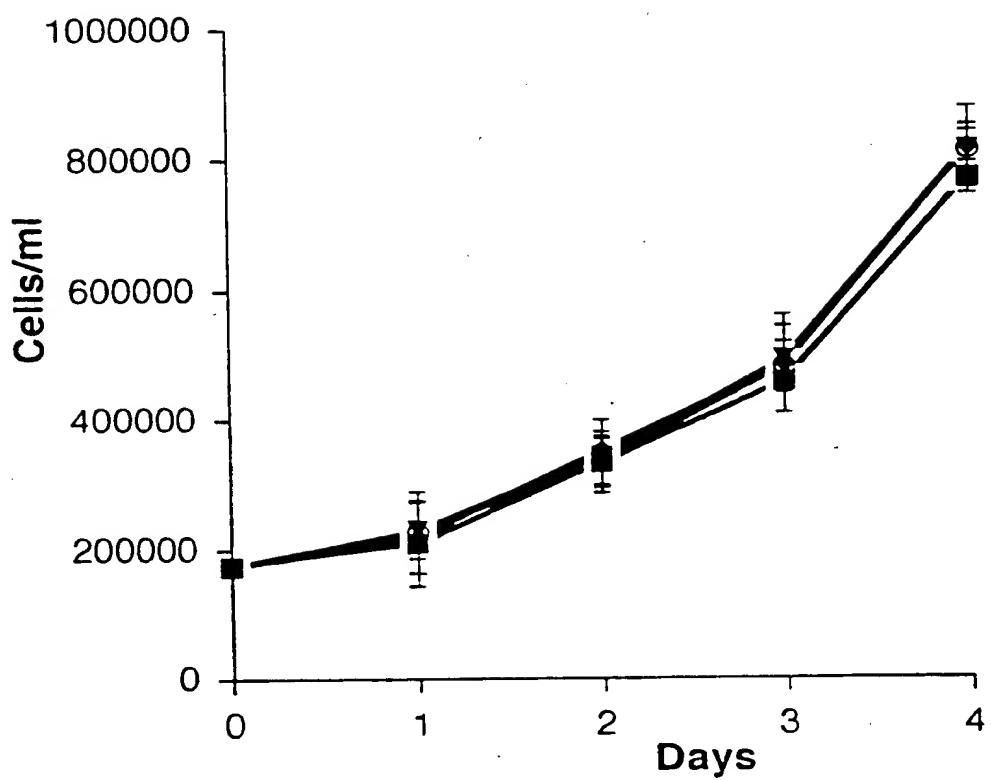
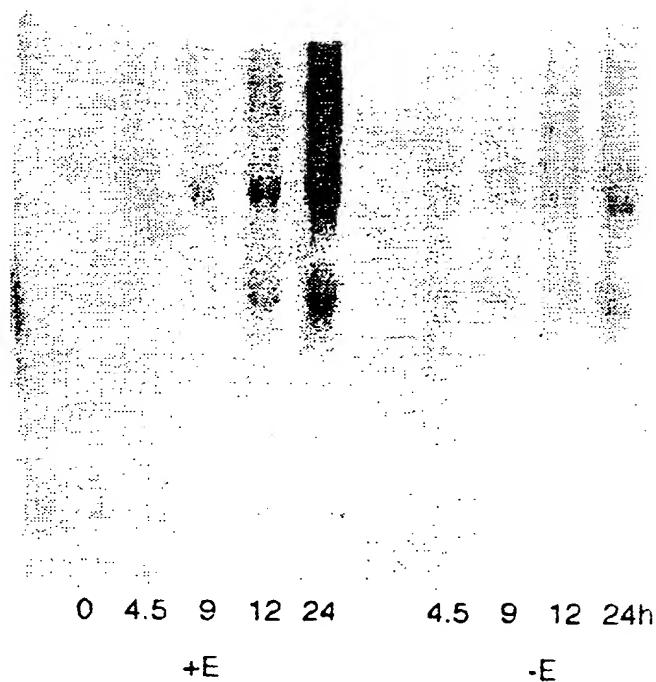


Fig. 12C

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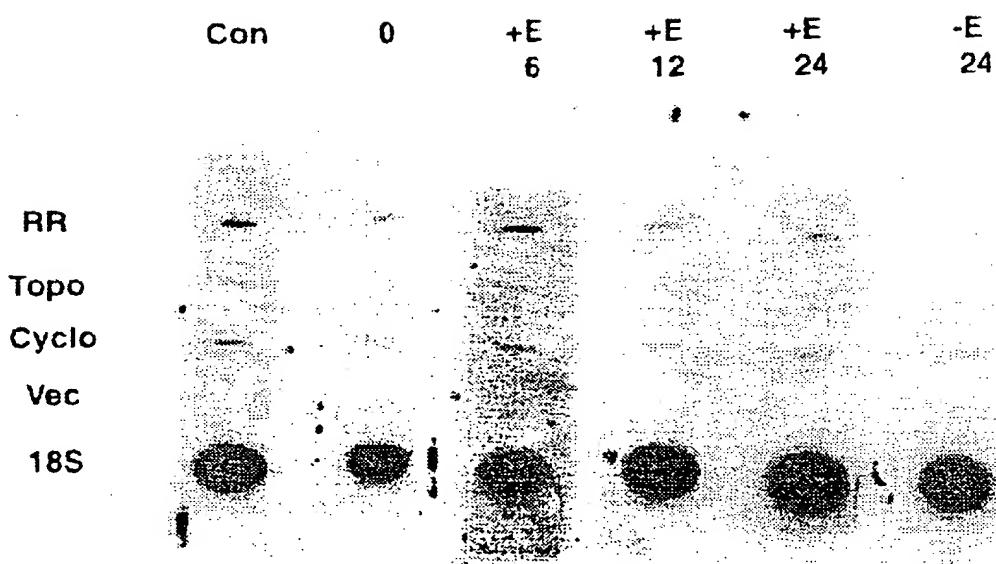
Fig. 13A



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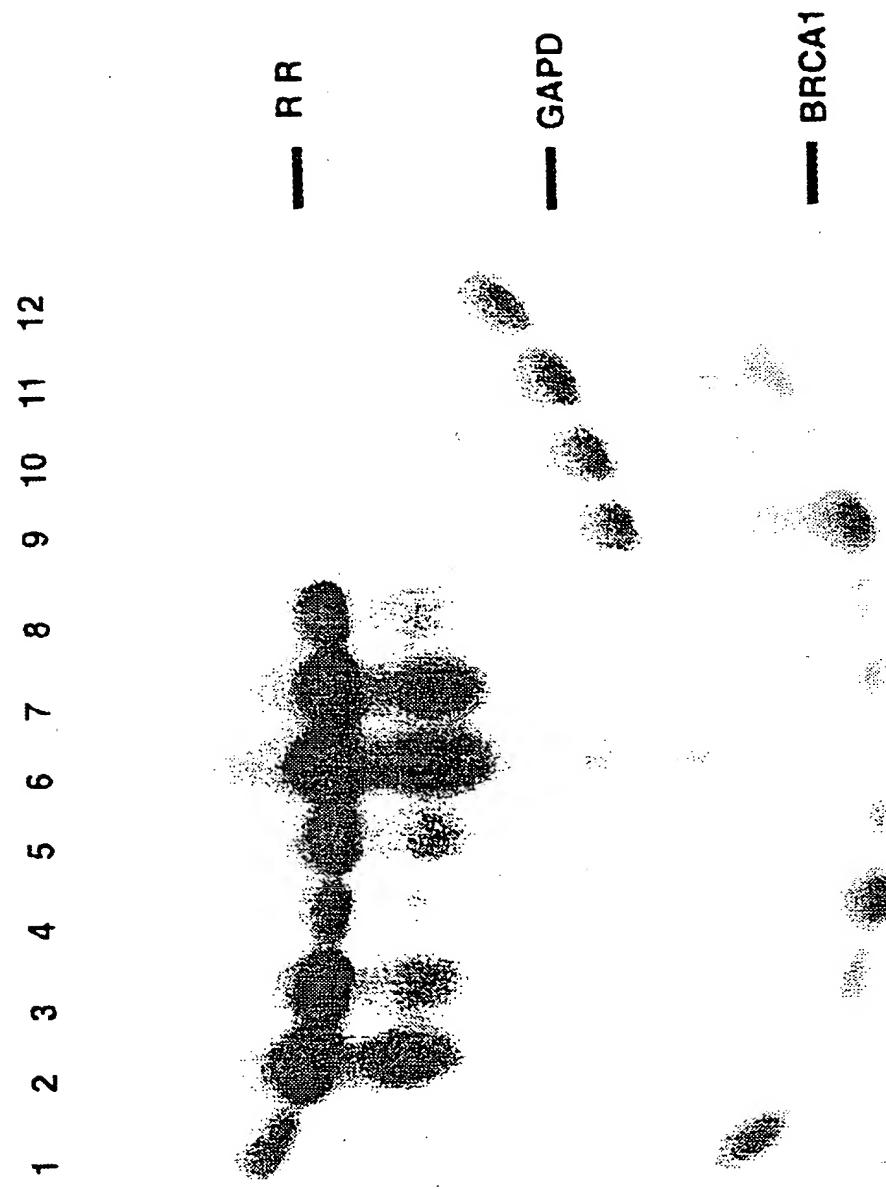
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Fig. 13B



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Fig. 14



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## INTERNATIONAL SEARCH REPORT

Int'l application No.  
PCT/US95/00608

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.2, 172.3, 320.1; 514/44; 536/23.2, 23.5; 935/3, 6, 9, 11, 14, 23, 77, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, Y	Science, Vol. 266, issued 07 October 1994, Y. Miki et al., "A Strong Candidate for the Breast and Ovarian Cancer Susceptibility Gene BRCA1", pages 66-71, see entire document.	1-13, 15, 18-37, 40
Y	Cancer Research, Vol. 52, issued 15 December 1992, P. Liang et al., "Differential Display and Cloning of Messenger RNAs from Breast Cancer versus Mammary Epithelial Cells", pages 6966-6968, see entire document.	1-13, 15, 18-37
Y	Cancer Surveys, Vol. 18, issued 1993, J. T. Holt et al., "Histopathology: Old Principles and New Methods", pages 1-16, Tables 1 and 2 and Figures 1-5b, see pages 7-12.	1-13

 Further documents are listed in the continuation of Box C.  See patent family annex.

Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier document published on or after the international filing date
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not to conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"Z"	document member of the same patent family

Date of the actual completion of the international search

21 APRIL 1995

Date of mailing of the international search report

04 MAY 1995

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer  
Stephanie W. Zitomer, Ph.D.  
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)\*

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/00608

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DNA (N.Y.), Vol. 5, No. 5, issued 1986, Neuhold et al., "Dioxin-Inducible Enhancer Region Upstream from the Mouse P-1450 Gene and Interaction with a Heterologous SV-40 Promoter", abstract, see entire document.	41-49
E, Y	US, A, 5,399,346 (ANDERSON ET AL.) 21 March 1995.	50-55

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

in ... nal application No.  
PCT/US95/00608

## Box I Observations where certain\claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-13, 15, 18-37, 40-55
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/00608

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 21/02, 21/04; C12Q 1/68; G01N 33/53; C12P 21/00; C12N 15/63, 15/85; A61K 48/00

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/6, 7.1, 69.2, 172.3, 320.1; 514/44; 536/23.2, 23.5; 935/3, 6, 9, 11, 14, 23, 77, 78

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG: Biosis, Derwent Biotech. Abstracts, WPI, Chem. Abstr., Diss. Abstr., Embase, Medline, Current Biotech. Abstr. (Royal Soc.); search strat: (cancer or carcinoma)(p)(breast or ovar?)(p)(gene or nucleic)(p)gene(..) sequence, nucleic(..) sequence?

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-13 and 25-37, drawn to a first process of using and a first product used: a diagnostic process using the product nucleic acids.

Group II, claims 15, 18-24 and 40, drawn to a second process of using, an immunoassay.

Group III, claims 38 and 39, drawn to a second product used, proteins including polypeptides and antibodies.

Group IV, claims 41-45, drawn to a third process of using, a process of screening compounds for activity in breast cancer treatment.

Group V, claims 46-49, drawn to a fourth process of using, a process for producing an indicator compound.

Group VI, claims 50-55, drawn to a fifth process of using, a process for treating breast cancer.

The inventions listed as Groups I-VI do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Groups III-VI lack the diagnostic technical feature and the inventions of Groups II-VI lack the nucleic acid special feature of Group I while the inventions of Groups II and IV-VI each have a different result such that they individually lack the special features of the others that are responsible for that result: The Group II process has a diagnostic result; the Group IV invention identifies a compound that affects the expression of the BRCA1 gene; the Group V process produces an indicator compound; the invention of Group VI treats cancer.

